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Translational Medicine
From laboratory discoveries to clinical practice

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Abstract:

Heliothis species are serious pests of agricultural crops including lettuce, cotton, sunflower, tomato, okra, maize, tobacco, pepper, and soybean. Four Heliothis species (Heliothis peltigera, Heliothis armigera, Heliothis viriplaca, Heliothis nubigera) which causes serious economic losses in every year are distributed in Turkey. Cultural methods, natural enemies and some chemical agents are used in the control of these pests. However, they are not effective enough for the control of pest. Baculoviruses are one of the natural microbial enemies of insects. They are large, complex DNA viruses with over 600 host species having been described until now. They are used broadly to control Heliothis species in the world. However, application of exotic virus strains may cause adverse effects on native strains; also exotic isolates may not be effective on native host in some conditions. Thus, it is important to identify native isolates that indicate high virulence in each geographical region and use them against native pests. In this study, we conducted a survey study to determine native and efficient virus isolates against Heliothis species in Turkey. Totally 1347 Heliothis larvae were collected from cotton, sunflower, aspir and chickpea fields in Southeastern Anatolia and Çukurova between 2014 and 2017. Twenty two larvae, indicating the signs of typical baculovirus infections, died within a few days. As a result of microscopic and molecular analysis, five baculovirus isolates from H. armigera (HearMNPV-O1, HearMNPV-O2, HearSNPV-S1), H. peltigera (HepeSNPV) and H. viriplaca (HeviMNPV) were determined. Among these, HepeSNPV and HeviMNPV were described the first time. Morphological and molecular features of all isolates were determined and dose response tests were performed in four Heliothis species distributed in Turkey and the results showed that HepeSNPV is a highly promising biocontrol agent against Heliothis species in the pest management. Key words: Baculovirus, microbial control, biopesticide, Heliothis.
IN-SILICO AND IN-VIVO MODELS FOR QATARI SPECIFIC CLASSICAL HOMOCYSTINURIA AS BASIS FOR DEVELOPMENT OF NOVEL THERAPIES

Nasrallah, Gheyath (1)

(1) Assistant Professor, Biomedical Science Department, Qatar University, Qatar.

Abstract:

Background: Homocystinuria is a rare inborn error of methionine metabolism caused by cystathionine θ-synthase (CBS) deficiency. However, in Qatar, the prevalence is 1:1800 births due to a founder Qatari missense mutation, c.1006C>T, where arginine (R) is replaced by cysteine (C) at CBS p.336 (R336C). Methods: We characterized the structure-function relationship of R336C mutation and investigate the effect of different chemical chaperones to restore R336C-CBS activity of using three models: In-silico, CBS yeast, and CRISPR/Cas9 R336C knock-in HEK293T and HepG2 cell lines. Results: Protein modeling suggests that R336C induces severe conformational and structural changes and thus could influence CBS activity. Complementation of CBS deficient yeast with a plasmid carrying wildtype copy of human CBS (p.hCBS), but not the R336C mutation (p.R336C), was able to restore the growth defect of CBS yeast. Treatments with chemical chaperones, proline and betaine, were able to partially restore the growth defect in the p.R336C CBS yeast. However, same chaperones treatment was only able to restore the stability and tetrameric conformation, but not the CBS activity in the R336C knock-in HEK293T cells. Conclusion: These results indicate that R336C mutation has a deleterious effect on CBS structure, stability, and activity, and chemical chaperones could be potential targets for treating this disease-causing mutation.

Attachment:

Pathogenic mutations in androgen receptor detected in Iraqi patients causing an accelerated growth of glioma cells in brain tumor

Hadi, Noora (1)

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Abstract:

A total number of 50 males with malignant brain tumors were selected and subjected to study the involvement of androgen receptor in glioma. Tissue samples were collected from Neuroscience Hospital in 2017 and subjected to molecular analysis using specific primers designed for this purpose. Testosterone levels were measured in all patients with glioma and significant change was found in their blood (p <0.001). DNA sequencing for the specifically amplified androgen receptor in tumor tissue revealed the presence of 79 pathogenic mutations. These included Androgen resistance syndrome, Hypospadias 1, X-linked, Partial androgen insensitivity syndrome, and Prostate cancer susceptibility. The last type of mutation may suggest that glioma in these patients are metastatic cells initiated from prostate. No obvious relation was found between glioma and testosterone receptor in tumor tissue which requires an extended study to establish if such connection is found.
** Gender similarities and differences in brain activation strategies  

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**Abstract:**  
Similarities and differences between males and females have been the subject of debate for more than a century (1). Recent development of functional brain imaging, particularly functional magnetic resonance imaging (fMRI), provided a major breakthrough to boost our understanding about these differences (2). Several previous studies aimed to study these differences during different tasks (3-5), but they were limited by the small sample size in each study. In this meta-analysis, the author studied brain activation during different task performance using fMRI. The author analyzed four main brain functions: Visual-spatial cognition, working memory, long term memory and emotion, each of which is represented by a specific test that is usually done during fMRI for each gender. The author utilized a systemic approach in his systemic review, where he searched studies manually through searching databases (i.e. Medline and Google scholar), and professional software designed for these purposes as shown in (supplementary material (6). From each study, we extracted coordinates for peak activation reported in (X, Y, Z). For each brain function, there are core areas that are used by both genders, as well as, gender specific areas that are activated exclusively in one gender. During complex thinking task, and in addition to the core areas, males activated their left superior frontal gyrus, compared with left superior parietal lobule in females. For memory tasks, several different brain areas were activated by each gender, which may reveal different strategies during short and long term memories. According to brain activation strategy in emotional tasks, valuation of emotional stimulus plays an important role in males’ strategy in emotional response, whereas females’ strategy is mainly affected by perception of the emotional stimulus.

**Reference:**  

**Attachment:**  
supplementary material:  
http://membs.org/membs/uploads/congress_speaker_files/1524945828supplementary material.docx
Biinformatics Analysis of Chronic Obstructive Pulmonary Disease (COPD)- Associated Interleukin-6 and CHRNA3 Genetic Variations

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Abstract:
Background: Chronic Obstructive Pulmonary Disease (COPD) is the fourth leading cause of death in the world. Genetics factors were found to contribute in the aetiology and progression of COPD. Recent studies demonstrated a significant association of Interleukin-6 (IL-6) and Cholinergic Receptor Nicotinic Alpha 3 (CHRNA3) genetic variants with increased risk of COPD in large case-control cohort. In-silico analysis showed that a Single Nucleotide Polymorphism (SNP) rs1818879 is located in the 3'untranslated region (3'UTR) of IL-6 gene and a synonymous SNP rs1051730 positioned in exon 7 of CHRNA3 gene. Objective: This report aims to use the available bioinformatics approach to investigate the potential functional effects of these COPD-related variants on the expression of IL-6 and CHRNA3 gene. Materials and Methods: Bioinformatics analysis of the IL-6 3'UTR SNP (rs1818879) using miRBase software was conducted to predict the micro-RNA (miRNA) binding to the target sequence. The possible impact of CHRNA3 silent SNP (rs1051730) on RNA secondary structure was evaluated using mfold software. Results: Our analysis showed that the alternative allele of SNP rs1818879 in '3UTR of IL-6 could disrupt the binding site of miRNA (hsa-mir-619-5p). With regard to CHRNA3 exon 7 SNP (rs1051730), the single nucleotide change at this location was predicted to cause a significant variation in the secondary structure of CHRNA3 protein-coding transcripts. Conclusion: The current study highlighted the possible role of regulatory and silent single nucleotide polymorphisms in disease pathogenesis. An extension of our work will include experimental validation using functional analysis.

Reference:

Attachment:
Bioinformatics Analysis of Chronic Obstructive Pulmonary Disease (COPD)- Associated Interleukin-6 and CHRNA3 Genetic Variations:

Comparison of Wild-Type and mutated CHRNA3-201 predicted mRNA secondary structures.:
http://membs.org/membs/uploads/congress_speaker_files/1525036533CHRNA3-201_dotted-1.png
“LHFPL5 mutation: a rare cause of non syndromic autosomal recessive hearing loss”

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(1) Senior Specialist, National Genetic Center, Royal Hospital, National genetic center, Oman.
(2) Senior Research Fellow, Leeds Institute of Biomedical & Clinical Sciences, St James's University Hospital, United Kingdom.

Abstract:

Hearing loss is a debilitating disorder that impairs language acquisition, resulting in disability in children and potential isolation in adulthood. Its onset can have a genetic basis, though environmental factors which are often preventable can also cause the condition. The genetic forms are highly heterogeneous and early detection is necessary to arrange appropriate patient support. Here we report the molecular basis of hereditary hearing loss in a consanguineous family with multiple affected members from Oman. Combining homozygosity mapping with whole exome sequencing identified a novel homozygous nucleotide substitution c.575T>C in the lipoma HMGIC fusion partner-like 5 gene (LHFPL5), that converted the 192nd amino acid residue in the protein from a leucine to a proline, p.(Leu192Pro). Sanger sequencing confirmed segregation with the disease phenotype as expected for a recessive condition and the variant was absent in 60,706 subjects from various disease-specific and population genetic studies as well as 50 unrelated individuals of Omani ethnicity. This study, which describes a novel LHFPL5 mutation in a family of Omani origin with hereditary hearing loss, supports previous clinical descriptions of the condition and contributes to the genetic spectrum of mutations in this form of deafness. This is the first report of a family from the Arabian Peninsula with this form of deafness.

Reference:

**Immuno-modelatory and biomolecule oxidation protective effects of Santolina chamaecyparissus ethanol extract**

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(6) Lecturer and Researcher, Department of Chemistry, Dicle university, Turkey.
(7) Lecturer and Researcher, Department of biochemistry, university batna 1, Algeria.

**Abstract:**

The immunomodulatory activity of ethanol extract (EE) of Santolina chamaecyparissus L. (S. chamaecyparissus) was evaluated by measuring the rate of IL-1beta and TNF-alpha released from concanavalin A-stimulated peripheral blood mononuclear cells (PBMCs) using ELISA method. Moreover, the protective effect against DNA and protein oxidative damage was investigated. Results showed that 100 µg/ml of EE of S. chamaecyparissus reduced TNF-alpha and IL-1beta release by 17.5% and 68%, respectively. On the other hand, DNA oxidation damage was inhibited dose-dependently in the presence of EE of S. chamaecyparissus. Indeed, at 0.5 mg/ml, the EE suppressed DNA cleavage by 89%. In the same manner, the protein oxidation was inhibited by EE of S. chamaecyparissus. At 1 mg/ml, EE protected protein fragmentation by 87%. The overall results suggest that EE of S. chamaecyparissus exerted antioxidant and immunomodulatory activities; hence it may serve as a potential source of natural antioxidants and anti-inflammatory compounds. Key words: S. chamaecyparissus, pro-inflammatory cytokines, PBMCs, DNA oxidation, protein oxidation.

**Reference:**

In Silico Analysis of Non-Synonymous Single Nucleotide Polymorphism in Human GGCX Gene Associated With Vitamin K-Dependent Proteins Deficiency

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(1) Lecturer, Haematology, Omdurman Ahlia University, Sudan.
(2) Lecturer, Biochemistry and Molecular Biology, University of Bahri, Sudan.
(3) Lecturer, Biochemistry and Molecular Biology, University of Bahri, Sudan.
(4) Head of department of biochemistry and molecular biology, Department of Biochemistry and Molecular Biology, University of Bahri, Sudan.
(5) Hassan, Genomics/Bioinformatics Unit, Africa city of technology, Sudan.

Abstract:
Genetic polymorphisms in gamma-glutamyl carboxylase GGCX gene have been associated with many vitamin-K dependent proteins disorders including vitamin-K dependent clotting factors deficiency, osteoporosis, vascular calcification disorders and PXE. Identifying functional SNPs in disease associated genes is difficult experimentally, so it's better to explore putative functional SNPs first. In this study various computational tools have been used to identify nsSNPs which are deleterious to the function, stability and structure of GGCX enzyme and might be causing these diseases. In silico analysis was performed using different bioinformatics tools include: SIFT, Polyphen-2, SNPs & Go, PhD-SNP and Provean to predict SNPs functional effect on the protein, while I-mutant and Mupro where used to check the stability of the protein upon SNP and Project Hope to predict SNPs structural effect. The study revealed five previously reported disease causing SNPs include [rs121909675 (L394R), rs121909677 (F299S), rs121909678 (G558R), Rs121909681 (R476C) and rs121909682 (R476H)] and four novel SNPs include: rs372264733 (L319P), rs146560494 (N388K) which are located in a domain that is important to protein activity, and rs185952482 (H287R) which disturb a stretch of repeated residues that are important for protein function, in addition to rs368938677 (Y379C) which is located in a transmembrane protein and may result in more hydrophobic protein which lead to loss of hydrogen bonds and may disturb protein folding. Future studies should consider these nsSNPs as main target in various diseases involve GGCX dysfunction. This is the first study where GGCX gene variants were analyzed using in silico tools hence will be of a great help while considering experimental studies and also in developing treatments for disease related to these polymorphisms. Furthermore mutational studies might be helpful in exploring the precise effects of these SNPs.

Reference:
Results of SNPs prediction of GGCX gene:

Attachment:
Levels of metabolic markers in drug-naive prediabetic and type 2 diabetic patients

KASABRI, VIOLET (1)

(1) PROFESSOR OF BIOMEDICAL SCIENCES, Biopharmaceutics and Clinical Pharmacy, UNIVERSITY OF JORDAN, Jordan.

Abstract:

Violet Kasabri1* [PRESENTER], Amal Akour1, Nailya Boulatoval1, Yasser Bustanji1, Randa Naffa1, Dana Hyasat2, Nahla Khawaja2, Ayman Zayed2, Munther Momani2. 1 Schools of Pharmacy and Medicine, the University of Jordan, Amman, Jordan 2 National Center of Diabetes, Endocrinology and Genetics, as well as Endocrinology and Diabetes Unit, the University of Jordan Hospital, Amman, Jordan *Address correspondence to: Violet Kasabri, email: hotice162@gmail.com AIMS: Type 2 diabetes mellitus (T2DM) and prediabetes (pre-DM) are associated with changes in levels of metabolic markers. The main aim of this study is to compare the levels of betatrophin, omentin, irisin, endothelin-1, nesfatin, hepatocyte growth factor (HGF), fibroblast growth factor, and oxytocin (OXT) between normoglycemic and pre-DM/T2DM obese Jordanian patients.

METHODS: One hundred and ninety-eight adult Jordanian subjects were recruited. Demographic data and clinical parameters were collected. The serum levels of biomarkers were measured by enzymatic assay procedure. RESULTS: Compared to normoglycemic (95 subjects), pre-DM/T2DM (103 subjects) displayed higher HGF (ng/ml) = 78.8 (71.4-104) versus 55.9 (45.3-66.6), p < 0.0001; and nesfatin (ng/ml) = 0.5 (0.4-0.7) versus 0.2 (0.1-0.4), p < 0.0001; betatrophin (ng/ml) = 1.2 (0.8-1.6) versus 0.22 (0.15-0.41), p < 0.0001. On the other hand, they had lower levels of omentin (ng/ml) = 2.1 (0.9-3.3) versus 3.6 (2.0-6.4), p < 0.0001, irisin (ng/ml) = 113.7 (88.9-142.9) versus 132.6 (110.7-147.8), p < 0.0001; and oxytocin (pg/ml) = 1077.9 (667.3-1506.0) versus 2180.1 (1464.5-2795.6), p < 0.0001; respectively. In comparison, FGF-21 (ng/ml) = 0.3 (0.2-0.5) versus 0.2 (0.1-0.4), and endothelin (pg/ml) = 2.7 (1.3-5.2) versus 2.8 (1.6-5.6) did not differ between the two groups (p > 0.05). Using stepwise multiple regression, OXT negatively and significantly correlated with HbA1c, FGF21, HGF but positively with both irisin and gender. TGs and HbA1c positively correlated, but irisin negatively correlated with betatrophin. Endothelin negatively correlated with irisin but positively with nesfatin. Nesfatin positively correlated with TGs, FPG, and HbA1c. FGF21 negatively associated with omentin and irisin. HGF negatively correlated with gender, OXT, and omentin but positively with age. Irisin negatively correlated with betatrophin, HbA1c, nesfatin, and FGF21 but positively with OXT, HDL-C, and age. Omentin negatively correlated with nesfatin, HGF but positively with irisin. OXT and irisin negatively correlated with HbA1c. Betatrophin positively correlated with HbA1c, while nesfatin positively correlated with FPG. Betatrophin and nesfatin positively correlated with TGs. CONCLUSIONS: In the present study, patients with pre-DM and T2DM have higher serum levels of metabolic HGF, nesfatin, and betatrophin and lower levels of omentin, irisin, and OXT. Future longitudinal and interventional studies are required to confirm the utility of these markers as novel progression or therapeutic targets in the pharmacotherapy of diabetes.
ROLE OF COMPLEMENT CASCADE IN STROKE

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(2) Research Assistant, Neurosurgery, Barrow Neurological Institute, United States.
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Abstract:

Brain cells may elicit secondary events, which can lead to cell death by inflammatory mechanisms. Among the myriad injury mechanisms involved in the pathogenesis of ischemic stroke, complement cascade activation plays a central role. Cerebral ischemia promotes complement cascade initiation and downstream anaphylatoxin generation incites a robust inflammatory response by priming ischemic endothelium and promoting the infiltration of inflammatory cells. Complement cleavage in stroke begins through the mannose binding lectin (MBL) pathway. Although circulating blood serves as the primary store of complement proteins, complement is also synthesized by resident brain cells including neurons and glia. This locally synthesized complement serves a dramatically different physiologic function than that described for circulating complement. Recently, a novel role for complement in developmental synaptic pruning was described, a process thought to be mediated by microglial phagocytosis. This same process has been shown to be aberrantly activated in several neurodegenerative diseases. As C1q is expressed in association with synaptic proteins in the peri-infarct region, this process of synapse elimination may also play a critical role in the pathogenesis of cerebral ischemia and stroke-related functional disability. We anticipate that C1q expression will be noted in close association with synaptic markers beginning early following stroke, with increased expression in the peri-infarct region at 24 and 72 hours. This synaptic localization of C1q classical pathway activation results in C3b opsonization of a subset of neuronal synapses in the subacute period of stroke. We also anticipate that activated microglia will engulf complement tagged synapses in the peri-infarct region in the subacute phase of stroke ischemia, as demonstrated by the presence of both complement and synaptic markers within the microglial cytoplasm, and C3 will be confirmed by analysis of synaptosomal fraction of tissue derived from the peri-infarct region. C3 antigen will co-localize with C1q at a delayed time-points suggesting that
The impact of rs1543297 and rs1800247 polymorphic sites of osteocalcin gene and its serum protein level in the development of metastatic bone tumors.

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(1) Consultant, Molecular Pathology and Genetics, Al Nahrain University, Iraq.
(2) Professor, Biotechnology, Genetic Engineering and Biotechnology Institute, Iraq.
(3) PhD student, Biotechnology, Genetic Engineering and Biotechnology Institute, Iraq.

Abstract:

Background: Osteocalcin is involved in bone calcification, resorption and remodeling. It may be specific in monitoring bone metastasis. Osteocalcin level in serum is affected by many factors including vitamin D, vitamin K, serum calcium and serum Alkaline Phosphatase enzyme. Bone metastasis can be of osteolytic nature like what happens in metastatic breast carcinomas or osteoblastic such as most of the prostate carcinomas. Objectives: The research aimed at determining the frequency of osteocalcin gene polymorphism at the rs1543297 and rs1800247 and serum osteocalcin in patients with metastatic bone tumors in comparison to controls. Methodology: A case control study was conducted between 40 patients with metastatic bone tumors and 40 controls. Patients were recruited at the Oncology Hospital of the Medical City Campus/Baghdad/Iraq. Genotyping of the candidate SNPs was conducted by Taqman based Real time PCR. Serum osteocalcin was measured by ELISA. Results: The homozygous mutated TT (52.5%) and the heterozygous CT(25%) genotypes of rs1543297 as well as the heterozygous TC (45%) genotype of rs1800247 were significantly associated with metastatic bone tumors, p<0.05. The mean level of serum osteocalcin in patients with cancer was 17.24±2.5 ng/ml and of the controls was 19.74±2.9 ng/ml. There was no significant difference between them. The mean level of serum osteocalcin in the homozygous mutated genotype group (15.76±1.3 ng/ml) was significantly lower than controls (27.4±9). Conclusion: Best to the present knowledge this is the first study in Iraq to deal with osteocalcin gene polymorphism and its protein product. The significant association of the mutated genotypes with the development of bone metastasis explains the high frequency of advanced stage solid cancers in Iraqi population.

Reference:

Regulation of Protein Kinase C ?II (PKC?II) gene expression in Chronic Lymphocytic Leukaemia (CLL) cells

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(1) Assistant Professor, Medical Laboratory Sciences, Al-Balqa Applied University, Jordan.

Abstract:
Chronic lymphocytic leukaemia (CLL) cells are derived from mature B lymphocytes and are distinctive with respect to overexpression of the classical protein kinase C isoform protein kinase C?II (PKC?II), which is encoded by PRKCB. Expression of PKC?II in CLL plays a vital role in the pathogenesis of the malignant cells in this disease, and within the microenvironment cells where it provides signals for the production of factors which support the survival of CLL cells. In CLL cells PRKCB transcription is stimulated by vascular endothelial growth factor (VEGF) through a mechanism involving activated PKC?II. However, the molecular regulatory mechanism(s) governing expression of the PKC? gene are poorly described. Thus, to characterise the factors regulating PRKCB transcription in CLL cells we used different approaches including mithramycin treatment, a drug which intercalates into GC-rich areas of DNA to inhibit binding of specificity protein 1 (Sp1), specific Sp1 siRNA, promoter function assays and site directed mutagenesis and chromatin immunoprecipitation (ChIP). Experiments using these techniques showed that Sp1 has a direct role in driving expression of the gene coding for PKC?II in CLL cells. Our results also show that Sp1 is highly associated with the PRKCB promoter in CLL cells compared to that in normal B cells, and suggest that this is likely because of the presence of histone marks permissive of gene activation. Besides, our results from pyrosequencing study demonstrated that there is no change in the methylation status of the CpG islands, which located near Sp1 binding sites within PKC? gene promoter between CLL and normal B cells. Examination of other transcription factors such as Sp3, MITF, RUNX1 and E2F1 that potentially bind the PRKCB promoter showed that they have static or indirect effects in regulating transcription of this gene. The exception to this is STAT3 which our data suggests plays a role in suppressing PKC? gene expression in CLL cells. Exploration of the mechanism through which VEGF induces PRKCB transcription revealed that this growth factor stimulates increased association of Sp1 and decreased association of STAT3 with the PRKCB promoter. Thus, VEGF-stimulated activation of PKC?II may play a role in this process. Taken together, Sp1 is the major driver for overexpression of PKC?II in CLL cells, and because this transcription factor is also overexpressed in these cells, the mechanisms we describe controlling PRKCB transcription potentially provide a foundation for further study of other genes contributing to the phenotype of CLL cells that are regulated by this pleiotropic transcription factor.
**Academic stress-induced changes in Th1- and Th2-cytokine response**

Assaf, Areej (1)

(1) Associate Professor, Biopharmaceutics and Clinical Pharmacy, The university of jordan, Faculty of Pharmacy, Jordan.

**Abstract:**

Psychological stress stimulates physiological responses releasing catecholamines and corticoids, which act via corresponding receptors on immune cells, producing a shift in the cytokine balance. These responses are variable depending on the nature of stressors. The effect of the academic stress on the production of the Th1-cytokines (TNF-?, IFN-?, IL-1?, IL-2, IL-6 and IL-8) and Th2-cytokines (IL-1ra, IL-4, IL-5 and IL-10) on 35 medical/health sciences students after completing their questionnaires was investigated. Blood samples were taken at three stages; baseline stage at the beginning, midterm and final academic examination stages. Plasma cortisol and cytokines were measured during the three stages. The last two stages were compared with the baseline non-stress period. Results of the stress induced during the final examination stage were the highest with a significant increase in cortisol release, IL-4, IL-5 and IL-1ra release with a shift in Th1:Th2 cytokines balance towards Th2. Whereby, the midterm stage did not show significant reduction in Th1-cytokines except for TNF-?, with an increase in IFN-? level that was reduced in the third stage. Th2 cytokine, IL-1ra, had positive correlations with Th1 cytokines; IL-2 and IFN-? in the second stage and IL-6 cytokine in the third stage. Cortisol was positively correlated with IL-8 in the last stage and heart rates had negative correlation with IL-10 in the first and last stages. Findings of this study indicate that exam stress down-regulates Th1 with a selective up-regulation of Th2-cytokines. In conclusion, Cortisol might have a role in suppressing the release of Th1-mediated cellular immune response which could increase the vulnerability among the students to infectious diseases.
Diversity of rotavirus genotypes circulating in Kashmir-Himalayan Population and impact of vaccination

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(1) Associate Professor, Genetics & Biotechnology, SK.University of Agricultural Sciences & Technology of Kashmir, India.
(2) Consultant, Department of Anaesthesia & Critical Care, King Fahads Specialist Hospital, Dammam, KSA, Formerly Department of Anaesthesia, GB Panth Children Hospital, Srinagar, Kashmir, India., Saudi Arabia.
(3) Consultant, Dept of Genetic Medicine, John Hopkins, ARAMCO, Dammam, KSA, Saudi Arabia.

Abstract:

The Kashmir has a temperate climate and incidence of rota viral disease is 45% under four years of age group. District Srinagar is an urban district with an estimated population of more than 1.3 million. Children between 0-6 years of age constitute about 14% of population (0.16 million). We estimated the impact 3600-3800 rotavirus vaccinations in private sector given in the form of two oral doses (Rotarix) per year from Jan 2013 to Jan 2015. Kashmir has an average health care setup where Jammu and Kashmir state has a birth rate of 18.3 per 1000 live births. Infant mortality rate is 37 and death rate is 5.3 per 1000 respectively. Better institutional delivery of immunization proves to reduce number of deaths due to diarrhea. Fully immunized children account for 66.6% and children under 3 years of age taken to health care facility are about 67%. Although use of ORT is not so appreciable yet 53% use ORT for diarrhea. Women on an average have a fair knowledge of diarrhoea (70%) and a health care facility is present after every 3 Km. Methods: Sample collection. RNA isolation and PAGE. RT-PCR for genotyping of the virus using 5 sets of primers (G- typing- 9 con1, 9 con2 G1,G2,G3,G4,G9 & G12) & (P- typing- con 3, con 2, P[8],P[4],P[6],P[9] & P[10]). Non-typeable samples were subjected to sequencing of VP7 gene and the genotype determined by comparison. Phylogenetic tree was constructed by clustal X software. The study was conducted on two groups I & II. Group I was non-vaccinated population randomly selected and included children admitted to children hospital for gastroenteritis. The children were screened for presence of rotavirus from Jan 2011 to Jan 2013. Group II was the vaccinated population receiving ( 2 oral doses of RV vaccine {Rotarix} at 4-8 weeks interval) from Jan 2013 -Jan 2015. Data was collected from the only children hospital of the valley. Results: Incidence of rota viral disease under 4 years of age group was about 45%. Rotavirus in group I was present throughout the year with winter peak from December to March (Mean 56.6%) followed by summer peak from April to June (Mean 42.4%) and autumn peak from August to November (Mean 23%). Incidence of disease in group I was: from 13 to 18 months (62.5%) followed by 7-12 m (59.12), 0-6 months (48.57%), 19-24 months (28.5%), 25-36 months (26%) and 37-48 months (22.83%). Exclusively breast fed were 47%. Food restriction in 15% cases with high rate of antibiotic use (65%). Continuation of breast feeding during diarrhea was about 84%. HR-Vaccine significantly reduced incidence of severe RV diarrhea in infants during first 2 years of age. A slight but significant decline in the admissions from urban areas of Kashmir valley was reported (10%).As the number of children vaccinated were quite less (7200 in two years), but the impact on overall cases of gastroenteritis was significant. G6 P types detected were G1,G2,G9 & G12 and P[4],P[6] & P[8] respectively. No case of vaccine induced intussusceptions was reported. Conclusion Vaccine is the best way to reduce the impact of morbidity and mortality of virus in inaccessible areas of poor health care and terrain. Swift and significant declines in hospitalizations were observed after immunization in urban Srinagar, Kashmir from private healthcare centers. Frequent use of ORT plus education on diarrhea prevention with zinc supplement and continuation of breast feeding is recommended. Since Govt. of India has rolled its rotavirus vaccine (Rotavac) in a phased manner in its national immunization programme, its impact along with other rotavirus vaccines will prove as a significant one to reduce overall disease burden from gastroenteritis. The decrease in rotavirus gastroenteritis was more in age group of less than 2 years, but was also evident in older children possibly due to herd immunity. RV vaccine efficacy may vary depending upon type of circulating strains and reduced efficacy to oral RV vaccine with high prevalence of malnutrition and gastrointestinal infections. Rich diversity of rotavirus strains circulating in India posses a question for evolution of more novel strains. Hence efforts for carrying surveillance on large scale population should be carried in order to access the percentage prevalence of strains in India in general & Kashmir particular- being a land locked Valley, high tourist influx (domestic, national and global) and annual pilgrimages in good numbers. Diagnosis of the rotavirus is rarely, if ever made at local level inspite of the fact that physicians & policy makers may appreciate that diarrhea is the first or second leading cause of death in children 5 years of age.
Activation of androgen receptor promotes breast cancer cell migration and invasion via metalloprotease 13

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Abstract:

Breast cancer can be classified according to their molecular profile into different types. The most common class is known as luminal breast cancer. The behavior of luminal breast cancer is dependent on the expression of steroid hormone receptors, particularly estrogen receptor and androgen receptor (AR). Although AR is considered a good prognostic factor, it correlates with increased invasiveness and is an activator of metastasis in breast cancer. Cancer cell invasion is facilitated by metalloproteases. Previously, we have observed that activation of AR induces the release of metalloprotease 13 (MMP-13) in molecular apocrine breast cancer cells. Using luminal T47D breast cancer cells, effects of DHT on cell behavior and MMP-13 expression were examined. Treatment of cells with AR hormone ligand, dihydrotestosterone (DHT), increased cell proliferation, motility, migration, and invasion. These effects were inhibited by treating cells with bicalutamide, an AR antagonist. In addition, DHT caused extensive alterations in the structure of the actin cytoskeleton and increased release of MMP-13. Interestingly, the effect of DHT treatment on cell migration and invasion, but not motility, was blocked by treating cells with a MMP-13 inhibitor. These results suggest that AR has important functional roles in the progression of luminal breast cancer, some of which are mediated by MMP-13.
Activation of androgen receptor induces epithelial-to-mesenchymal transition of molecular apocrine breast MDA-MB-453 cancer cells: Alterations of a molecular network

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Abstract:
Epithelial-to-mesenchymal (EMT) is an early event that occurs as cancer cells progress from a carcinoma in situ stage into the more aggressive invasive characteristic. In fact, EMT is quite complex involving many orchestrated cellular and molecular alterations in cancer cells. We have observed that treatment of molecular apocrine breast MDA-MB-453 cancer cells, also known as a luminal androgen receptor (AR) model system, with AR ligand, dihydrotestosterone (DHT), induces in what appears to be a mesenchymal phenotype. This alteration is associated with a change in the actin cytoskeleton and increased cell migration, both of which are inhibited by bicalutamide, an AR antagonist. The changes are more prominent after 3 days of treatment with DHT. Exposure of cells to DHT for a few hours is sufficient to induce the changes in cell morphology and actin cytoskeleton after 3 days with the maximal effect of 16 hours of exposure. These results indicate that EMT is induced as a result of altered gene expression. Investigation of changes in the expression of 84 EMT-related genes revealed up-regulation of the genes expressing Slug and regulator of G-protein signaling protein 2 (RGS2) and down-regulation of those expressing β-catenin and Transcription Factor 4 (TCF4). Further analyses confirmed the latter results at the protein level. AR is also found to interact with β-catenin, Slug, and RGS2 proteins. However, whereas AR-Slug interaction is stimulated following AR activation by DHT, AR dissociates from RGS2 in the presence of DHT. In addition, knocking down expression of Slug abolishes DHT-induced EMT of the cells. On the other hand, cells transfected with RGS2 siRNA results in EMT-like phenotype in the absence of DHT, and intriguingly, the cellular alteration is abolished upon inhibiting AR. Furthermore, DHT augments the phenotypic change in RGS2-knockout cells. Interestingly, both Slug and RGS2 also interact with each other. However, this interaction becomes weaker with increasing duration of exposure to DHT. These results indicate that AR induces EMT in luminal AR cells via altering a network of signaling molecules with roles of Slug mediating AR action and RGS2 blocking it.
Clinical Exome Sequencing Unravels Novel Disease Causing Genes and Mutations in Highly Consanguineous Middle Eastern Families with Suspected Mendelian Disorders from Qatar.

Al-Dewik, Nader (1)

(1) Consultant Clinical Scientist, Pediatrics department, Hamad Medical Corporation, Qatar.

Abstract:

Background Mendelian disorders in consanguineous populations are not only common but may be diagnostically challenging due to the increased probability of novel alleles expressing themselves atypically as well as dual molecular diagnoses. Clinical exome sequencing (CES) lends itself readily to these challenges. Methods We performed CES in 508 probands referred to the Clinical and Metabolic Genetics at Hamad Medical Corporation-Qatar from April 2014 to December 2016. Importantly, CES was a first-tier molecular test in the majority of cases. Results Of the 508 patients enrolled, a clear genetic diagnosis (pathogenic or likely pathogenic mutation relevant to the phenotype) was made in 242 (47.6%), consanguinity and positive family history were associated with a higher diagnostic yield reaching up to 56% (Odds Ratio: 2.16 [95% CI, 1.2-3.6], P =0.02). A dual or triple molecular diagnosis was identified in 35 (7%) of the cohort. Two homozygous mutations in the same gene, compound heterozygous variants/mutations and copy number variants were identified in 3 (0.5%), 13 (2.57%), 4 (0.7%) respectively. An apparently recessive mutation in genes hitherto only linked to dominant phenotypes was identified in two cases. We also highlight interesting variants in 23 novel candidate genes, which could explain the clinical presentation but require additional confirmation. Interestingly, the diagnostic rate was found to be significantly higher in the singleton - CES 84/131 (66%) cases vs trio-CES 36/71 (50%) (Odds Ratio: 1.7[95% CI, 0.96- 3.1], P < 0.04) in children aged from 6 to 18 years than others. Reanalysis of “negative” cases revealed 30/124 (24%). Most families opted not to receive ACMG secondary findings but among those who agreed, only one had such a finding. Conclusion We attribute the high diagnostic rate we observed in this study compared to other studies in part to the high rate of consanguinity and our reanalysis of “negative” cases in light of newly published literature. Our data corroborate a growing body of evidence in support of considering CES as a first-tier molecular test in patients with suspected Mendelian phenotypes.

Attachment: 
Abstract :
Classical Homocystinuria in the Qatari Population: Clinical, Biochemical and Molecular Characterizations

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(1) Consultant Clinical Scientist, Pediatrics department, Hamad Medical Corporation, Qatar.

Abstract:

Background: Classical Homocystinuria (HCU) is the most common inborn errors of metabolism in Qatar with an estimated incidence of 1 in 1800 newborns due to consanguinity and founder mutation R336C in the cystathionine β-synthase (CBS) gene. The objective of this study is to describe the phenotype of this severe devastating B6 nonresponsive HCU. Method: A single center study was carried out between 2016 and 2017 with a total of 126 Qatari patients (54 female, 72 male from 57 families) with HCU. Detailed clinical and biochemical data were collected, Stanford-Binet Intelligence Scales), Quality of life (PedsQL) and adherence to treatment (Morisky scale) assessments were carried out prospectively. Patients were stratified into three groups according to mode of diagnosis: 1) Late Diagnosis Group (LDG), 2) Family Screening Group (FSG), and 3) Newborn Screening Group (NSG). Results: 69 patients (54%) are LDG, 44 (35%) patients are from NSG and 13(10%) patients are from FSG. Furthermore, the diagnosis for these patients has been confirmed by presence of homozygous R336C founder mutation. The main manifestations that led to the diagnosis in LDG were ocular manifestations (47 %), intellectual disability (ID) (37.2 %), thromboembolic event (5.8 %), seizure (5 %), and hyperactivity and behavioral changes (3.9%). Interestingly, fair hair and brittle nails were observed in (6 %) of LDG at diagnosis. During the disease course in LDG, 100% developed Marfanoid Habitus and osteoporosis, 92.1 % developed ocular complications, 82.3 % developed ID, 23.5 % had fractures,,23.53% had psychiatric problems, 17.6% had cardiac complications, 17 % developed hypertension 11 % had thromboembolic events and 5.8 developed severe neurological impairment and died at age of 18--30 years. Other rare complications include diabetes mellitus 4 %, bronchiectasis 4% and gastrointestinal bleeding 2% were observed in the LDG. In the FSG, 28.5% developed bilateral lens dislocation, 28.5% had cardiac abnormalities and 14 % developed fracture. Biochemical studies showed that homocysteine and methionine levels in LDG were significantly higher than in NSG and FSG (p<0.01). On the other hand, 64 % of NSG patients were classified as adherent to treatment and diet while 63% and 87% in FSG and LDG were non-adherent respectively (p<0.01). The range and median of IQ were (39 -113, 79) in LDG, (84 -110, 96) in FSG and (89- 116, 98) in NSG (p<0.01) and the median of QoL in LDG, FSG and NSG were 86%, 91% and 97% respectively (p<0.001). Conclusion: Our data shows a direct association between time of diagnosis and the outcomes of HCU with emphasis that early detection by NBS and early treatment significantly improves the outcomes of HCU. This study further contributes to a better understanding of the natural history of classical HCU.

Reference:

Not applicable

Attachment:
The abstract with author list:
The biostability of gold nanoparticles for cancer treatment

Hassan, Heba (1)

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Abstract:

Abstract Nanotechnology is the latest technology in this century where it has a wide field of applications. One important application can be found in medicine. Metal nanoparticles, such as gold, has been used in the treatment of cancer [1]. The cancer-treating gold nanoparticles (GNPs) have different chemical and physical synthesis methods. These methods can sometimes be expensive, toxic and produce uncontrolled size of gold nanoparticles[2]. This research aimed at the synthesis of GNPs using an eco-friendly method. The characteristics of the produced GNPs were studied to ensure their suitability in photo-thermal therapy. Additionally, the biostability of the GNPs were investigated. GNPs were synthesized using green method in which the reducing agent was black seed extract and the stabilizing agent was Gum Arabic. The synthesized GNPs were characterized utilizing UV-visible spectroscopy, transmission electron microscopy and X-ray diffraction. Then the biostability of GNPs was assessed using UV-Visible spectroscopy of submerged nanoparticles in bovine serum albumin, L-Histidine, L-Cysteine and normal saline solutions over a period of 15 days. The effects of different concentrations and volumes of reducing agent were monitored by forming low concentration (0.13 g/ml with volumes of 5, 10, 15, 20 and 30 ml) and a high concentration (0.53 g/ml with volume of 10 ml). The synthesized GNPs with 30 ml of reducing agent were found to have high crystalline structure at planes (111), (200), (220) and (311) with diffraction angles of 38°, 44.1°, 64.5° and 77.5°, respectively. The most abundant shapes formed by these gold nanoparticles were found to be spherical and hexagonal and the size for the same sample was in the range of 15-40 nm. The stability of gold nanoparticles was studied in sample made of 10ml high concentration reducing agent and obtained an approximately stable wavelength with slightly shift about ± 5 nm. GNPs were synthesized using a non-toxic, fast, low cost and eco-friendly method which make it preferable for medical applications such as cancer treatment.

Reference:


Attachment:

research discussion:
http://membs.org/membs/uploads/congress_speaker_files/1527499205research discussion.pptx
Evaluation of immunomodulatory effects of lamotrigine in BALB/c mice

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Abstract:
Modulation of the immune system has recently been shown to be involved in the pharmacological effects of old antiepileptic drugs and in the pathogenesis of epilepsy. Therefore, the most recent guidelines for immunotoxicological evaluation of drugs were consulted to investigate the immunomodulatory effects of lamotrigine, a newer antiepileptic drug, in BALB/c mice. These included the in vivo effects of lamotrigine on delayed-type hypersensitivity (DTH) response to sheep red blood cell (SRBC) antigens, hemagglutination titer assays and hematological changes. In vitro effects of lamotrigine on ConA-induced splenocyte proliferation and cytokine secretion were assessed. The results showed that lamotrigine treatment significantly increased the DTH response to SRBC in the mouse model of this study. This was accompanied by a significant increase in relative monocyte and neutrophil counts and in spleen cellularity. Lamotrigine significantly inhibited ConA-induced splenocyte proliferation in vitro and it significantly inhibited IL-2 and TNF-α secretion in ConA-stimulated splenocytes. In conclusion, the results demonstrated significant immunomodulatory effects of lamotrigine in BALB/c mice. These data could expand the understanding of lamotrigine-induced adverse reactions and its role in modulating the immune system in epilepsy.
Testing siRNA Design Hybridization Thermodynamics With off-Target Effect for Clinical Trials Enhancement.

Salem, Nourah (1)

(1) Fresh Graduate, Zewail University of Science and Technology, Zewail City of Science and Technology, Egypt.

Abstract:

In the coming paper, a computational study using The thermodynamic parameter (free energy) is applied on designed siRNA sequences. The study investigates the siRNA self folding, siRNA-target gene hybridization and siRNA-off target hybridization free energy. Here we mathematized base-pairing stability and measure the partition functions of these investigations. Through a comparison between our calculations and the obtained from another experimental previous work that used different parameters for determining siRNA efficiency, We can conclude whether our parameter (measurement of free energy binding) is a valid one that could allow for designing siRNA and quantify the contribution of off-target effect as well as the effect of self loops by either the siRNA or the target mRNA. A review of the basic criteria for designing siRNA computationally and the potential for clinical use is presented as well.

Reference:


Attachment:

Hybridization Examples Between siRNA with target: http://membs.org/membs/uploads/congress_speaker_files/1527507454rna.ps

Hybridization Examples Between siRNA with off-target: http://membs.org/membs/uploads/congress_speaker_files/1527507454AK000313.fasta

siRNA self loop1: http://membs.org/membs/uploads/congress_speaker_files/1527507454dot.ps

Review of the potential molecular mechanisms responsible for the direct cardiac effects of SGLT2R inhibitors.

Akour, Amal (1), Abbate, Antonio (2)

(1) Associate Professor, Biopharmaceutics and Clinical Pharmacy, The University of Jordan, Jordan.
(2) Associate Chair of Research, Division of Cardiology, VCU Pauly Heart Center, United States.

Abstract:

Introduction: Diabetes mellitus is a chronic disease (1) affecting 415 million patients around the world (2). Due to the complex nature of diabetes mellitus, therapeutic approaches to manage this illness do not only aim at glycemic control, but also to prevent and to slow the progression of microvascular complications; including retinopathy, nephropathy and neuropathy, as well as macrovascular complications, namely cardiovascular disease (CVD). Diabetes is one of the major contributable factors for CVDs and people with diabetes are 2-4 times more likely to die for heart diseases than their nondiabetic counterparts (1). Sodium-glucose transporter 2 receptor (SGLT2R) inhibitors are class of recent antidiabetic medications (3) which act by inhibiting the SGLT-2 receptors in the proximal convoluted tubule (4). This in turn will enhance glucose excretion in urine and reduce plasma glucose. Dapagliflozin and canagliflozin were the earliest members to be approved in the US and Europe (4). Empagliflozin is one of the newest inhibitors that is more selective to SGLT2 receptors than other members (4). Clinical studies showed that these medications are effective in lowering glucose and have low risk of hypoglycemia. In addition, they exert various pleiotropic effects beyond glucose control such as reduction of blood pressure and body weight (3, 5). Of interest, the EMPA-REG OUTCOME trial showed that empagliflozin could reduce hospitalization due to heart failure by 35%, and cardiac-related mortality (RRR=32%) in patients with type 2 diabetes mellitus (T2DM) (6). Furthermore, results from the Canagliflozin Cardiovascular Assessment Study (CANVAS) (7) showed that canagliflozin, compared to placebo, reduced hospitalization due to heart failure by 33%, and also reduced mortality due to cardiovascular causes by 22%, nonfatal myocardial infarction, or nonfatal stroke by 14%. Indeed, EMPA is currently being recommended by the European Society of Cardiology for diabetic patients with heart failure, in combination with metformin (8). Several attempts to interpret the mechanisms behind these cardioprotective effects of SGLT-2R inhibitors were undertaken. These drugs have shown to modify cardiovascular risk factors in diabetic patients beyond glucose control such as reduction of blood pressure, arterial stiffness, body weight, visceral obesity, uric acid levels and oxidative stress (4, 5,9, 10).The later could be due diuretic effect, natriuretic effect (9), which consequently reduces cardiac preload and blood pressure. Also, it can be attributed in part (9), to decreased glucose toxicity and the enhancement of ketone oxidation (11). Nevertheless, the EMPA-REG Outcome trial showed that there was no concomitant reduction in stroke or myocardial infarction, which would have been expected to be the case through the effect of empagliflozin on blood glucose, blood pressure and/or body weight. In addition, HF outcomes did not correlate with glycemic control, as both the 10 and 25 mg dose have comparable effects on HbA1c, also, HbA1c reduction was not associated with the extent of benefit (10).

Expression studies showed that SGLT-2R, is almost exclusively expressed in renal cortex, and no expression was detected in heart tissues (12). Therefore, it is reasonable to speculate that SGLT-2R inhibitors might have a direct effect on the heart via off-target mechanisms or receptors. The aim of this review is to summarize preclinical studies which evaluated the direct cardiac effects of SGLT2R inhibitors in normoglycemic animal models. Methods: Literature search using PubMed and Google Scholar. Results and conclusions: The intriguing results of EMPA-REG Outcome and CANVAS trials strongly suggest that SGLT2R inhibitors can have cardioprotective effects beyond what would be merely expected from glucose control in patients with heart failure. This can be also devised from the currently available evidence of preclinical trials in normoglycemic animals where empagliflozin and canagliflozin could reduce cardiac hypertrophy and other markers of heart failure such ANP and BNP. Of interest, these effects also were brought about at clinically relevant concentration of the drugs and even after short interval of treatment (for e.g. 2 weeks). Nevertheless, few studies evaluated the potential mechanistic pathways responsible for these effects. One study suggested the role of NHE1 inhibition by empagliflozin on controlling the sodium and calcium overload, and expectedly enhancing the antioxidant machinery of the cells, which would protect the heart tissue from oxidative stress, continuous depolarization and ultimately heart failure. Although current studies did not investigate the downstream effects of NHE1 suppression by SGLT2R inhibitors, this can be extrapolated from studies that have already shown the anti-ischemic, anti-hypertrophic properties of NHE1 inhibitors such as cariporide and enporide. Moreover, the weak inhibitory effects of empagliflozin on cardiac SGLT1R could contribute, at least in part, to these effects, but this is only a hypothesis and needs further investigation. Clinical studies to assess the benefits of SGLT2R inhibitors in HF patients without diabetes are ongoing, but these studies will not provide mechanistic insights about the cardioprotective effects of SGLT2R inhibitors. Further in vitro, and preclinical studies are recommended to assess these mechanistic pathways. An animal model of SGLT2R knockout/down would be useful to understand the role of off-target receptors of SGLT2 inhibitors.

Reference:

glucose transporters (SGLT) in human ischemic heart: A new potential pharmacological target. International journal of

Impairment of Cardiac Glucose Uptake by Phlorizin during Ischemia-Reperfusion Injury in Mice. PloS one.

Kashiwagi Y, Nagoshi T, Yoshino T, Tanaka TD, Ito K, Harada T, et al. Expression of SGLT1 in Human Hearts and


effects of the Na(+)/H(+) exchange inhibitor cariporide in patients with acute anterior myocardial infarction undergoing


**microRNA Expression in Ethnic Specific Early Stage Breast Cancer: an Integration and Comparative Analysis.**

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(3) Professor, Biology, American University of Beirut, Lebanon.

**Abstract:**

In Lebanon, breast cancer (BC) is the most common cancer type among women, and has a higher incidence in younger patients compared to the West. microRNA (miRNA) are small noncoding RNA that act as master player at all stages of BC development. We recently showed that certain miRNA expression in Lebanese BC tissues was different than that reported in Western patients. This could reflect an ethnic difference and a necessity of global miRNA profile of Lebanese early stage BC tissues. Hence, the aim of this study is to examine miRNA expression in Lebanese BC tissues using microarray, perform comparative miRNA profile analysis with matched American samples and predict the role of dysregulated miRNA in early BC through mRNA-miRNA integration analysis. 74 miRNAs were differentially expressed in 45 tumor versus 17 normal adjacent breast tissues that were confirmed using reverse transcription real time PCR. Differences in dysregulated miRNA were noted in the comparative analysis that could reflect either patient age at diagnosis (miR-1288-star/3196), or ethnic variation in miRNA epigenetic regulation (miR-31/362-3p/663/329/22/373/320a/34b/196a/149/203) and sequence variation of precursor miRNA (miR-196a/185/206). mRNA profiling showed that BC samples were mainly of luminal B subtype. mRNA-miRNA integration analysis identified 719 potential mRNAs targeted by 51 miRNAs, with miR-183 and miR-182 having the highest number of targets. Integration also revealed the potential miRNA role in early BC by regulating tumor suppressive or oncogenic mRNA involved in increasing proliferation and decreasing migration and invasion. Furthermore, since miR-183 was among the most upregulated miRNA in the Lebanese patients, we investigated its role in BC development in vitro. Using in silico tools, targets of miR-183 were predicted: AKAP12 (A kinase anchor protein 12), PDCD4 (programmed cell death 4), TET1 (dioxygenase gene), SMAD4 (SMAD Family Member 4), PP2CA (serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform), FOXP1 (Forkhead box protein P1) and LATS2 (Serine/threonine-protein kinase). We found that transfection with miR-183 mimics inhibits the expression of AKAP12, PDCD4, TET1 and PP2CA mRNA in BC cell lines (MCF-7 and MDA-MB 231) without any significant change in cell proliferation. Our data provide a basis for genetic/epigenetic investigations to explore the role of miRNA in early stage BC in young women, including ethnic specific differences and further functional studies are being performed to identify the molecular mechanism of miR-183 on BC development.
Adenosine Diphosphate Receptor Gene (P2Y 12) Sequence Variants among Swedish, Palestinians and Congolese

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Abstract:
Keywords: P2Y 12, single nucleotide polymorphisms, H2 haplotype, Introduction: P2Y 12 receptor plays a central role in platelet aggregation and thrombus formation. Recently, inter-individual variations in platelet response of healthy untreated individuals were established which was explained by genetic variations in P2Y 12 receptor gene. Several single nucleotide polymorphisms (SNPs) in P2Y 12 receptor have been associated with increased platelet reactivity and risk of cardiovascular diseases. Therefore, this study aimed to evaluate the pathological H2 haplotype (using G52T as a tag-SNP) and 18C>T polymorphisms in three different ethnic groups; Palestinians, Swedish and Congolese. Methodology: The H2 haplotype and 18C>T SNPs were determined in conveniently selected healthy individuals from different ethnic groups (n=254). The whole exon-3 of P2Y 12 was sequenced and analyzed by used ABI PRISM 310 Genetic Analyzer. The major and the minor allele frequencies of the P2Y12 SNPs were determined in the study population and the genetic differences between ethnic groups in P2Y 12 were elucidated. In addition, the frequency of the genotypes were calculated among the ethnic groups. Results: In this study, five benign single nucleotide polymorphisms (SNPs) were genotyped and identified; 18C>T, 36G>T, 162G>T, 546C>T and 989A>G. The overall frequencies of each SNP in all study population (n=254) was 21.9, 10, 0.4, 0.6 and 0.4%, respectively. The frequency of H2 haplotype among Swedish (n=55), Congolese (n=54) and Palestinian (n=145) was 23.6, 12, and 4.1%, respectively, while the frequency of 18C>T was 20%, 6.5% and 28.3%, respectively. There were significant differences in frequency of H2 haplotype and 18C>T among the ethnic groups (P<0.001). In regard to the pathological SNPs, all of the study participants were negative. Conclusions: There are significant differences in the frequencies of the genetic variants of the P2Y 12 exon-3 between the study ethnic groups. Further studies should be performed to study the effect of the genetic variations effect on ADP or TRAP-induced platelet aggregation.
Using Human Induced Pluripotent Stem Cells (iPSCs) to Model Ataxia with Oculomotor Apraxia Type 1 Caused by Nonsense Mutation in the APTX Gene.

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(1) Assistant Researcher, Cell Therapy Centre, University of Jordan, Jordan.

Abstract:

Ataxia is a rare condition with a prevalence of 18.5 in 100,000 individuals. To date, there are more than 30 autosomal dominant forms of ataxia and over 60 forms of either autosomal recessive or X-linked diseases. Ataxias with oculomotor apraxia (AOAs) are the most common recessive ataxias with childhood onset and typically present with characteristic eye movement defect. Ataxia with Oculomotor Apraxia, Type 1 (AOA1) is characterized by early-onset cerebellar ataxia, usually less than 10 years of age with motor symptoms, slow progression, oculomotor apraxia and polyneuropathy. AOA1 is the most common recessive ataxia in Japan and the second most frequent in Portugal after Friedreich’s ataxia. To date, no disease-specific treatments are available for AOA1. AOA1 is related to mutations in the aprataxin (APTX) gene, located on chromosome 9p13.3 and consists of seven exons, which encodes aprataxin protein. The aprataxin protein has been reported to be involved in DNA single-strand break repair (SSBR) machinery. Alternative splicing in exon 3 generates two distinct isoforms, the longer transcript encodes for a 342 amino acid protein, while the shorter one encodes a 174 amino acid protein. It has been shown that cells deficient in aprataxin are sensitive to genotoxic agents that cause single strand breaks in DNA. In addition, an increase in chromosomal aberrations and DNA breaks were also observed in camptothecin-treated AOA1 cells compared with controls. Almost all of the identified mutations affect the HIT hydrolase domain localized in exons 5, 6 and 7, the most frequent being nonsense mutations. Fibroblasts from patients with AOA1 are hypersensitive to oxidative damage than normal fibroblasts and that an increase in oxidative DNA damage was observed in the cerebellum of AOA1 patients. Several recessively inherited truncation mutations have been reported in APTX gene, which suggests that the neuronal degeneration of AOA1 may be related to a loss of the protein function. Using whole-exome sequencing (WES), we were able to identify a nonsense mutation in APTX gene in two unrelated consanguinous Jordanian families, one family has four affected siblings and the second family has more than 25 patients carrying a homozygous APTX mutation. This mutation, c.837G>A is predicted to result in a stop-gain mutation (Trp279X) in the aprataxin protein. This mutation was previously found in several AOA1 patients of European origin. Mutation analysis in the relatives confirmed that the parents of all affected families were heterozygous while the unaffected siblings are either heterozygous or wild type. We have also assessed the patients clinically and they all showed consistent characteristics of AOA1, such as motor disability, severe weakness and wasting and discoordinated eye movements. The access to the affected human brain neuronal cells is essential for understanding the cellular and molecular mechanisms driving the AOA1 disease. Human postmortem samples have been used to study neurological diseases phenotypes. However, these samples represent only the end stage of the disease and are difficult to attain. Transgenic and knockout mouse models can provide valuable information about genetic disorders in vivo and in vitro. However, mouse models do not fully recapitulate human diseases. Furthermore, the mouse brain does not faithfully reflect the human brain anatomically and developmentally, thus some brain regions commonly affected in cerebellar ataxia do not have counterparts in the mouse models. To help translate research from animal models into novel therapeutic strategies for ataxia patients, it is essential to validate disease pathophysiological findings in the relevant affected human cell types. The invention of iPSC cellular reprogramming has revolutionized human cellular disease modeling in vitro. Somatic cells such as fibroblasts can be reprogrammed into a pluripotent state by the overexpression of defined transcription factors (Takahashi & Yamanaka 2006; Takahashi et al. 2007). The iPSCs are highly similar to human ESCs, with the ability to indefinitely proliferate and differentiate into any cells derived from the three germ layers. iPSCs technology would allow the generation of personalized human neurons from AOA1 patients to study the pathophysiology underlying the APTX mutations. These iPSCs are patient-specific with the advantage of retaining the genetic background of the same patient, to recapitulate the cellular phenotypes of patient’s neurons in vitro. Additionally, they provide a platform to test new drugs and genetic therapies as well as a source of cells that could potentially be used for cell replacement therapy in the future. In this project, we aimed to reprogram AOA1 patient fibroblasts carrying the APTX mutation into iPSCs and to further differentiate them into mature neurons, to develop neuronal model system to study AOA1 disease pathophysiology. Our established iPSC lines displayed a morphology and cell characteristics similar to ESCs. The iPSCs formed tightly packed and flat colonies with large cellular nuclei and scant cytoplasm when cultured on layer. All iPSC lines were transferred to feeder-free conditions on Matrigel and maintained in mTeSR1 medium. Control iPSC lines were chosen by matching age and gender with our patient lines. Three iPSC lines were generated for each patient, expanded and banked in LN for long-term storage to give rise to stable cell lines. The isolated iPSC lines were extensively characterised, based on their morphology and ability to express pluripotency markers, karyotyping results, Sendai virus clearance and Mycoplasma testing result. Thus the generation of these iPSC lines would allow us to investigate the contribution of this mutation to disease pathogenesis, studying oxidative stress, sensitivity to DNA-damaging agents and genome instability in AOA1 iPSC-derived neurons. Theses iPSCs-derived neurons would provide the opportunity for novel drug discovery in the future using high-throughput drug screening platforms and for identifying new disease targets for testing the efficacy and toxicity of...
drug-based therapies. Few studies have generated iPSC-based models of the cerebellar ataxias and to the best of our knowledge, this study is the first to generate iPSC models for APTX-related AOA1 disease.

Reference:

Identifying two gene clusters that may serve as gene signature and biomarker of Atopic Dermatitis (AD) and can predict pimecrolimus efficacy and pharmacoepidemiology

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(1) assistant professor, department of clinical pharmacy, isra university, Jordan.

Abstract:

Introduction: Calcineurin antagonists (pimecrolimus) treat atopic dermatitis (AD) with different effectiveness and adverse events. Objectives: We wish to characterize and build a network to serve as gene signature of Atopic Dermatitis (AD) and also to predict pimecrolimus efficacy and pharmacoepidemiology. Materials and Methods: gene expression profile were taken from GSE32473 gene expression profile of patients' skin suffering from AD. The samples were ten baseline AD patients, and ten AD patients after topical treatment with either pimecrolimus. A total of 72 genes were found with unique expressions patterns in contrast between AD patient's baseline and patients receiving pimecrolimus. geneMANIA, online tools was deployed to find the relevant network and subnetwork clusters. Results: geneMANIA database search yielded 49% in co-expression pattern. 27% overlaps in pathway networks between the submitted genes. Four genes distinctly involved in regulation of receptor activity. Another four were in regulation in IkappaB kinase signaling. Differentially expressed after administration of pimecrolimus. Conclusions: Analysis of differentially expressed genes after pimecrolimus reveals two significant gene clusters that are involved in receptor and Kappa kinase signaling pathways. These genes may function as biomarkers for pimecrolimus effectiveness and adverse events.

Reference:


Attachment:


Studies on antimicrobial activities of extracts from wild and in vitro grown cultures of Solanum villosum

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(2) Professor of plant biotechnology and biodiversity, Department of Horticulture and Agronomy, Faculty of Agriculture, University of Jordan, Amman, Jordan, The University of Jordan, Jordan.
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(4) Researcher, Agricultural and Animal Research Center,Tripoli, Libya,, Agricultural and Animal Research Center,Tripoli, Libya,, Libyan Arab Jamahiriya.

Abstract:

Solanum villosum is a wild medicinal herb belongs to family Solanaceae. Although it has been classified as a weed, Solanum villosum has many medicinal properties with major components of alkaloids. Few research had studied Solanum villosum antimicrobial activities of extracts taken only from wild plants. So, with such medicinal value there is a need to better understand anti-microbial activities of this plant. In this study, in vitro cultures of microshoots and callus of this plant were multiplied on Murashige and Skoog media, supplemented with different growth regulators. Extracts from each tissue cultured plant material and from wild plants were screened using both Disk Diffusion and Microdilution Assays (MIC) for their antibacterial and antifungal potential and were compared with antibiotic activity against the tested microbes. For Antibacterial activity; a promising data of growth inhibition were obtained from in vitro grown microshoots of Solanum villosum which were very closed to antibiotic and wild plant extract results. For disk diffusion assay; the most inhibited bacteria strains were, Klebsiella pneumoniae and Staphylococcus epidermidis with growth inhibition zones diameter of (30, 25 mm; respectively) using microshoots extracts. Meanwhile, callus extract was less effective in antibacterial activity than microshoots and resulted in maximum inhibition zones of (20, 18.7 mm); recorded in (Klebsiella pneumoniae and Staphylococcus epidermidis; respectively). In MIC method; microshoots extract was effective at MIC values of (9.77 and 39.06 (µg/ml) against (Micrococcus luteus ; Klebsiella pneumoniae ; respectively). On the other hand; for antifungal activity of Solanum villosum; The inhibition zone of Candida albicans by callus extract using disc diffusion assay was ( 10 mm) diameter and MIC value was 9.7 (µg/ml). While, for wild plant extract the inhibition of growth zone of Candida albicans was (20 mm) diameter and MIC value was 4.9 (µg); which were very closed values to callus extract results. From this study, we can conclude that in vitro grown Solanum villosum plant has a promising effective antimicrobial activity to the most strains tested compared to the wild plant. This can be utilized to maximize the production of the active ingredients in this plant in vitro which might facilitate production of natural antibiotics with antimicrobial activity closed to manufactured antibiotics.
A molecular approach for evaluation of experimental trials of anti schistosomal vaccination in murine models

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Abstract:

Current schistosomiasis control strategies are mainly based on chemotherapy, but many researchers believed that the best long term strategy to control schistosomiasis is through immunization with antischistosomiasis vaccines. This work is concerned with assessment of the efficacy of different potential anti-schistosomal vaccines (as crude soluble egg antigen (SEA), soluble worm antigen preparation (SWAP) and combined SEA & SWAP) by parasitological and molecular studies in experimental murine models. Sixty male laboratory bred Swiss Albino mice were used and divided into six groups; control normal (G1), control infected by 80 ± cercaria by S.C. route (G2), Freund’s adjuvant (adj.) received then infected (G3), SEA+adj. received then infected (G4), SWAP+adj. received then infected (G5) and combined (SEA+SWAP) + adj. received then infected (G6). A schedule of sensitization, immunization and schistosomiasis challenge was followed and performed on different mice gps. Mice were euthanized 10 weeks post-infection. Potential vaccines efficacy was investigated by parasitological and molecular studies including egg count/gram stools using modified Kato thick smear, liver egg load, oogram pattern in the liver and stools, PCR to detect S. mansoni egg DNA in stools of studied mice. Results showed that the combined (SEA + SWAP) vaccine caused the highest significant reduction in the fecal egg count followed by SWAP then SEA antigens while, the highest percentage reduction of eggs/gram liver tissue was attributed to (SEA+SWAP) followed by SEA then SWAP antigens. Regarding oogram results, the combined (SEA + SWAP) antigens were more efficient in increasing the number of dead ova with highly significant reduction in the number of mature & immature ova, followed by SEA then SWAP antigens. The lowest percentage of S. mansoni egg DNA detected by PCR in stools samples was found with the combined (SEA + SWAP) antigens, followed by SEA then SWAP antigens. So, PCR assessed the combined antigens (SEA + SWAP) as the most effective, they presented the highest grade of protection towards schistosomiasis challenge, this was manifested by the lowest percentage of S. mansoni egg DNA in stools. The parasitological and molecular studies results were nearly similar but the molecular study was more sensitive, definite and accurate.

Reference:


Introduction: Disrupted intracellular iron metabolism in cancer increases the generation and propagation of reactive oxygen species and the oxidative stress that associates with that. Increased oxidative stress has been implicated in the initiation and progression of different forms of cancer including breast and colorectal cancers. In this study we investigated the expression status of seven key proteins involved in intracellular iron metabolism (Hepcidin, Hemeoxygenase-1, Transferrin receptor 1 and 2, Ferroportin, Ferritin and Catalase) in 57 clinical samples obtained from different patients with different forms of colorectal cancer (CRC) and in seven CRC cell lines. Materials and methods: In this work we have extensively studied the expression of 7 iron regulatory proteins; Transferrin Receptor 1 and 2, Ferroportin, Ferritin and Catalase. In addition, we analyzed 2 more proteins that interacted with them, hepcidin and hemeoxygenase-1. The investigation was done on a group of 57 formalin-fixed, Paraffin-embedded colon cancer tumor tissue samples and 7 colorectal cancer cell lines with varying clinical and pathological characteristics using immunohistochemistry (IHC), and Western Blot (WB) respectively. For functional analysis, siRNA silencing for Ferroportin was done followed by investigating the expression change in Hepcidin and Ferroportin. Results: Iron regulators expression in human colon cancer sections were as follows; Ferroportin, TR1, catalase and Ferritin respectively showed elevated expression with Ferroportin showing the highest frequency; (97%) of the total number of samples showed positive expression, while 51% of tumors showed weak positive ferritin expression. TR2 was moderately expressed in 32% of CRC samples. The application of statistical analysis on CRC samples revealed that Catalase correlated significantly with TR1 and TR2. While, no significant correlation was found between ferritin and any of the analyzed iron regulators. Catalase expression levels and different age groups showed significant correlation in a cluster analysis. Regarding iron regulators expression in human colon cancer cell lines, Catalase had dramatic variation in expression levels. Two primary cancer cell lines, HT29 and HCT116, showed the highest fold increase in Catalase expression, 6.2 and 4.6 folds respectively. TR1 was found to be highly over expressed in the cell line of primary CRC with Wild type P53 the rest of the cell lines had mutated P53 and showed significant lower levels of TR1 expression levels. The colon epithelial cell line with wild type P53 showed the lowest level of TR1 expression. TR2, on the other hand, had no dramatic increase in expression compared to the colon epithelial cells. Hepcidin (Hep) was found to have an inversely related expression with Ferroportin in all CRC cell lines regardless of their stage or pathological characteristics with no constant ratio. All CRC cell lines had high hepcidin content that was dramatically higher than Ferroportin content in any of the investigated CRC cell lines. Also, all had marked increase in the expression of and hemeoxygenase-1 (HO-1). Cell lines derived from late stage cancer showed the highest HO-1 expression levels, with above 30-fold increase in comparison to normal epithelia, showing consistently high expression with the positive control for HO-1 which is breast cancer MCF7. In comparison to normal colon epithelia, Ferroportin showed high fold increase in expression levels in all colon cancer cell lines except for one cell line which is derived from a primary tumor of an early stage, had Ferroportin expression level similar to the normal epithelial. The silencing of Ferroportin; caused Hepcidin levels to rise up reaching 3-folds of increase, while Ferritin levels slightly increased in comparison to the negative controls. Discussion: All investigated iron regulating proteins showed elevated expression in CRC compared to normal epithelial cells, making them good markers for CRC. Elevated TR1 and TR2 along with down regulated Ferritin, suggest that increased iron uptake and reduced iron storage may contribute to iron overload. However, as most histological samples used were of late stage, we cannot conclude on the role of these markers in early detection of CRC. Ferroportin and HO-1, may be of limited applications in CRC as they did not show differential expression in primary CRC cell lines compared to normal. On the other hand, Hepcidin showed dramatic increase in expression making it a good marker for CRC and particularly this might be applied in early stage detection. In conclusion: these findings suggest that colorectal cancer associates with disrupted iron metabolism and that it shows significant heterogeneity in the expression profile of key iron regulatory proteins. Understanding the underlying mechanisms of disrupted iron homeostasis in colorectal cancer and how it influences disease progression and treatment is work in progress.

Reference:

The Use of Next Generation Sequencing and Array CGH data in the diagnosis of genetic disorders in Jordan, case studies

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(1) Head of Molecular Genetics Unit, Molecular Genetics Laboratory, Department of Laboratory Medicine and Pathology, Specialty hospital, Jordan.
(2) Assistant Professor, faculty of pharmacy, Petra University, Jordan.

Abstract:

Next-generation sequencing (NGS) and microarray have been significantly contributed to the transformation of genomic research by providing access to the genome for analysis, by significantly decreasing the sequencing costs and increasing the throughput. The next goal is to exploit this powerful technology in the clinic, namely for diagnostics and therapeutics. NGS and array provides a lot of possible clinical applications, the potential of some of the current NGS systems to transition to the clinic, the identification of causative mutations for rare genetic disorders through whole-genome or targeted genome resequencing, the application of those technologies for family genomics, and NGS data analysis tools. Here, we will discuss some array CGH & NGS data for genetic disorders and how we succeeded in the utilisation of those data in the diagnostic services and genomic medicine in Jordan, mainly in heterogeneous group of diseases such as leukodystrophy and muscle diseases.
Characterization of genetic alterations in Lymphoblastic leukemia:
advances and impact in clinical management.

Chaker, Hend (1)

(1) Assistant Professor (Assistante Hospitalo-universitaire), Cytogenetic and Molecular Genetics, Institute Pasteur, Tunisia.

Abstract:
Thanks largely to translational research, leukemia diagnosis; prognosis and treatment have made huge progress over the last decades. Since the identification of the translocation, t(9;22)(q34;q11), in 1960, as non-random genetic alteration associated to Chronic Myeloid Leukemia (CML), several other molecular markers have been identified in different types of leukemia. The discovery of these genetic alterations, not only allowed many discoveries in genetic mechanisms underlying normal hematopoiesis and leukemogenesis, but also offered interesting biomarkers for leukemia diagnosis, prognosis and target therapy. Since 2001, World Health organization (WHO) classification of Tumors of the Hematopoietic and Lymphoid Tissues, included genetic anomalies in the classification of Acute Lymphoblastic Leukemia B (B-ALL). Translocations t(9;22)(q34;q11), t(4;11)(q21;q23), t(12;21)(p12;q22), t(1;19)(q23;p13.3), Hyperdiploidy and Hypodiploidy are among the most recurrent well defined genetic biomarkers, revealed by karyotype, Fluorescent In Situ Hybridization (FISH) or aCGH techniques. The detection of those biomarkers is becoming a routine in the laboratories of Medical Centers treating leukemia, to allow leukemia risk stratification, choice of treatment and bone marrow engraftment indication. Through genomic analysis advances, emerging biomarkers have been identified, and recently “BCR-ABL1-like” B-ALL subclass was integrated in the last 2016 WHO classification. “BCR-ABL1-like” B-ALL is characterized by gene expression profiling similar to B-ALL presenting BCR-ABL fusion gene, with activation of ABL or JAK-STAT Kinases pathways, caused by different genetic alterations. Leukemia is particularly interesting example that research results have influenced consistently the clinical management of affected patients. Here, we present results of our research findings in characterizing genetic alterations associated with B-ALL and review the different technologies breakthroughs (from karyotype to CGH array and Next Generation Sequencing (NGS)) and their impact in leukemia patients management.
WHOLE EXOME ANALYSIS OF OMANI PATIENTS WITH
PREDOMINANTLY NEUROLOGICAL DISORDERS

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(2) Clinical Molecular Geneticist, Genetics, Sultan Qaboos University Hospital, Oman.

Abstract:

Whole-exome sequencing (WES) is increasingly being used as a first-line test in clinical practice for the diagnosis of Mendelian diseases. Here we describe the diagnostic yield and molecular findings of locally-performed WES and analysis in Omani families. We initiated a WES program (Jan 2015- Dec 2017) in a cohort of Omani patients of predominantly pediatric age. WES was performed on the locally-available Ion Proton platform within the Next-Generation Sequencing Core Facility. Analysis and annotation of exome data were performed following a simple in-house pipeline. Variants were computationally filtered based on allele frequency, pathogenicity scores, genic position and zygosity. Candidate gene variants and segregation analysis in available family members were performed by Sanger sequencing. Neurologic manifestations included developmental delay, intellectual disability, ataxia and seizures. Most patients had undergone multiple genetic and other laboratory investigations prior to WES (so-called diagnostic odyssey). Molecular diagnoses were reached in 63% (17/27) of cases studied, which is higher than that reported in the literature. As expected given the prevalence of consanguinity, variants segregated in a recessive manner in most families, but also included de novo and X-linked inheritance. Our experience demonstrates that whole exome sequencing on the locally-available Ion Proton is feasible and is yielding a molecular diagnosis in a substantial proportion of Omani patients with a neurological phenotype. Establishing the molecular basis has important implications for clinical management and recurrence counseling in at-risk relatives. Even in the presence of consanguinity, additional mode of inheritance beyond recessive must be considered during exome data analysis.
**Molecular Diagnostic Testing of Solid Tumors from International Guide Lines to Local Practice**

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(1) Clinical Scientist, Diagnostic Genomic Division, Hamad Medical Corporation, Qatar.

**Abstract:**

Introduction: Molecular genetics biomarkers are widely used as diagnostic, prognostic and predictive tool for many cancer conditions. For several cancers, established guide lines are involve the molecular biomarkers which improve the cancer patient’s management and also increase the clinical impact of response to treatment. Aim: To set up molecular testing work flow according to standard guide lines to improve local services for cancer patients. To introduce molecular testing for detection of somatic variants in DNA/RNA extracted from formalin fixed paraffin embedded (FFPE) tissue collected from cancer patients. Method: Different technologies were used to establish cancer testing services according to standards set by the Collage of American Pathologist (CAP). These techniques include Real Time – PCR and BIOCARTIS, Sanger sequencing and next generation sequencing (NGS). A Testing strategy was developed to improve the turnaround time (TAT) to overcome the limitation of each technique and to provide comprehensive testing services. Results: Testing for several genes that contain molecular biomarkers were successfully implemented in Diagnostic Genomic Division (DGD) at Hamad Medical Corporation (HMC). These genes are KRAS, BRAF, NRAS, EGFR, cKIT, PDGFRA and IDH1 & IDH2. Based on our validation study, limit of detection for each used method was as follow: Real Time – PCR and BIOCARTIS with up to 1%, Sanger sequencing 20% and NGS 5%.Patients with several cancer types, including colon, lung, brain, melanoma and GIST were beneficiary of this service. Since November 2016, approximately 300 cases were tested; the highest referrals were for lung and colon cancer. The TAT reduced from 1 moth to 2 days for some tests. Conclusion: Providing molecular testing for cancer patients have improved the clinical outcome by providing results in short TAT especially for patients who needed targeted therapy.

**Reference:**

Increased expression of Meteorin-like hormone in Type 2 diabetes and obesity

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(1) Research associate II, Department of biochemistry and microbiology, dasman diabetes institute, Kuwait.

Abstract:
Background and Objectives: Type 2 diabetes (T2D) is a growing pandemic associated with metabolic dysregulation and chronic inflammation. Meteorin-like hormone (METRNL) is a recently identified adipomyokine [1]. Animal studies revealed that METRNL is secreted from skeletal muscles in response to physical exercise and promotes energy expenditure and thermogenesis in adipose tissue [2]. MTRNL has also been linked to T2D, where it has been shown to accelerate insulin resistance, hyperinsulinemia and hyperglycemia [3]. Our objective was to study the changes in the level of METRNL caused by T2D and obesity in our population and to assess the association of METRNL level with irisin. Methods: In this study, A total of 228 individuals were enrolled including 124 non-diabetic (73 non-obese and 51 obese) and 104 T2D (38 non-obese and 66 obese) individuals. Plasma level of MTRNL and irisin were assessed using ELISA. Statistical analysis was performed using the Mann-Whitney U test considering P < 0.05 as significant data. Pearson correlation was used for association analysis. Results: The results showed that plasma level of METRNL and irisin were significantly higher in T2D patients (1263.52 ± 24.96 pg/ml and 623.0± 21.8 pg/ml respectively) compared to non-diabetic (1198.58 ± 24.28 pg/ml for METRNL and 513.6 ± 16.1pg/ml for irisin) p-Value < 0.05. When the population was further stratified based on obesity METRNL and irisin level were significantly higher in obese T2D (1311.88 ± 32.1 pg/ml and 668.1 ± 24.3 pg/ml respectively) compared to non-obese T2D individuals (1179.52 ± 36.1 pg/ml and 547.1 ± 39.2 respectively) p-Value < 0.05. No significant difference was observed between obese and non-obese non-diabetic individuals for both proteins. Additionally, METRNL level was significantly correlated with BMI (r2 = 0.196, P = 0.003) and Irisin level (r2 = 0.233, P = 0.001). No significant correlation was observed between METRNL and TG, HDL, LDL and TC. Conclusion: Our data shows that METRNL plasma level was increased in T2D patients and was further exacerbated in patients with obesity. Also, from the correlation analysis the data suggests a possible interplay between METRNL and Irisin specially that both are adipomyokines that play a role in the regulation of beige fat thermogenesis. Further studies are required to examine the role that METRNL plays in T2D and obesity as well as how the changes in its level may affect other myokines such as irisin.

Reference:
Abstract:
Progress of nanotechnology in medical arena has fueled a burgeoning interest in optimal use of nanoparticles in cancer therapy. Metal oxide, particularly ZnO NPs have been widely explored as a versatile platform for targeting cancer cells with highest specificity and therapeutic efficacy. In the present study, cytotoxicity of two sets of nanoparticles, set 1 containing native and copper doped ZnO NPs (ZNP1, ZNP2, ZNP3) and set 2 containing doxorubicin loaded particles (ZNP4, ZNP5, ZNP6) was investigated against cancer cell lines (HeLa and MCF-7) and normal cell line (HCEC). ROS generation, apoptotic DNA damage and lipid peroxidation were assessed through respective bioassays. All nanoparticles showed remarkable anticancer activity as compared to the conventional chemotherapeutic doxorubicin (Used as positive control). Doxorubicin loaded nanoparticles (set 2) presented a synergistic effect and their cytotoxicity increased with copper dosage. MCF-7 was found more sensitive than HeLa. ZNP6, a doped ZnO-Dox nanocomplex was the strongest particle tested. Moreover, significantly differential response among cancer cell lines (HeLa and MCF-7) and HCEC demonstrated their preferential cell killing potential. It was also found that ZNPs induced apoptotic cell death by ROS mediated DNA damage and lipid peroxidation. Hence, copper doped zinc oxide nanoparticles alone as well as in synergism with conventional chemotherapeutics can serve as a practical therapeutic modality with a strong potential to become a clinical reality.
Combined therapy of oncolytic Newcastle Disease Virus and rhizomes extract of Rheum ribes enhances cancer virotherapy in vitro and in vivo.

Al-Shammari, Ahmed (1), Abdul Jalill, Raghad (2), Hussein, Mohammed (3)

(1) Principal Investigator, Experimental Therapy Department, Iraqi Center for Cancer and Medical Genetic Research / Mustansiriyah University, Iraq.
(2) Professor, Biology, College of Science, Mustansiriyah University, Iraq.
(3) Msc student, Biology, College of Science, Mustansiriyah University, Iraq.

Abstract:

Phytotherapy has been used to treat a different type of diseases including cancer for a long time, and it was a source for different effective anti-tumor agents. Oncolytic Newcastle disease virus (NDV-ICCMGR-NAJAF) are very promising anti-tumor therapy (1). Nevertheless, NDV-based monotherapeutics have not been very effective to some resistant tumors. Thus, the efficiency of oncolytic NDV must enhance by combining NDV with other novel therapies (2, 3). The current study aimed to determine the possibility of improving the oncolytic effect induced by NDV through Rheum ribes rhizomes extract (4) administration in vitro and in vivo. Methods, the in vitro study include exposure of the crude extract of R. ribes alone or NDV alone or combination of both agents for 72h. The cancer cells tested were murine mammary adenocarcinoma AMN3, Human Rhabdomyosarcoma RD, and Human Glioblastoma AMGM5 (5), and using rat embryo fibroblast REF as normal control cells. MTT cell viability assay was used and analyzed for possible synergism using Chou-Talalay analysis method. In vivo experiment included study the combination and the modalities in the transplanted murine mammary adenocarcinoma AM3 line (6) and tumor sections analyzed by histopathology. Results, Combination therapy of NDV-R. Ribes showed enhanced oncolytic activity on cancer cells. With no cytotoxicity on normal cells. In vivo study showed that monotherapeutic modalities had low growth inhibitory effect on transplanted tumors in mice (37.036 to 32.46) %, extract and NDV respectively, but combination therapy showed 95.637 % growth inhibition, this had confirmed by histopathological examination that revealed the wider area of necrosis. In conclusion, the novel combination recommended for clinical application for breast cancer therapy.

Reference:

Anti-Inflammatory, Anti-Viral and Cytotoxic Activity of Honey Bee Venome

Salama, Mohammed Abdallah (1)

(1) Master Student, Molecular biology, GEBRI, Egypt.

Abstract:

Anti-Inflammatory, Anti-Viral and Cytotoxic Activity of Honey Bee Venom: Mohamed A Salama, Mohamed A Younis, Roba M Talaat Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City Background: Honey bee (Apis mellifera) venom (BV) or apitoxin therapy is part of a larger medical philosophy and treatment called apitherapy. It has been used to treat several inflammatory conditions. Recent studies have reported that BV can cause growth arrest and it has a direct cytotoxic effect on several types of cancerous cells. The present study aimed to evaluate anti-cytotoxicity, anti-apoptotic, antiviral and anti-inflammatory properties of BV as well as changes in cytokine secretion levels and nitric oxide (NO) production using three different cancer cell lines (Hep-G2, MCF-7 and HPV-18 infected HeLa cells) and 2 normal cells (splenocytes and macrophages (MQ)). Materials and Methods: MTT assay was used to assess the cytotoxic effect of BV against all tested tumor cells in addition to splenocytes and macrophages at different time intervals (24h, 48h, and 72h). Tumor necrosis factor (TNF-α), Interleukine (IL-10) and interferon (IFN-γ) were determined by enzyme-linked immunosorbent assay (ELISA). Nitric oxide (NO) was estimated by colorimetric assay. Caspase 3 expression was confirmed by reverse transcription-polymerase chain reaction (RT-PCR). Results: Our results showed a significant cytotoxic effect inducing death of different tested cell lines in a dose-dependent manner with a maximum cytotoxicity at 40 µg/ml. The cytotoxic effect of BV is widely different by time from one cancer cell type to another. Despite the cytotoxicity observed for BV in relation to tumor cell lines, none of the used concentration was toxic for normal cells. On the contrary, cell viability was increased in a dose-dependent manner. A reduction in IL-10, elevation in TNF-α with no change in IFN-γ was observed in HepG-2. MCF-7 cancer cells treated with 40 µg/ml BV has low IL-10 and TNF-α and high IFN-γ production level. On another hand, elevation of IL-10 and IFN-γ coincides with reduction in TNF-α level was demonstrated after incubation of HeLa cells with 40 µg/ml. The expression of Caspase 3 was dramatically increased with increase in BV concentration in all tested cancer cell lines with maximum expression at 40 µg/ml. Exposure of MQ to different BV concentrations showed gradual reduction of NO with increasing BV dose. A significant diminution in NO production was demonstrated at 40 µg/ml. Conclusion: Taken together, the results of this study stressed on the importance of BV as a potent anti-tumor and anti-viral agent with valuable anti-inflammatory activities. Further steps towards the use of bee venom for pharmacological purposes must be done.
Novel Mutation in the Mt-CO3 gene and 12s rRNA associated with Optic Neuropathy. 1 Dilshad Abdulla; 2 Shaho Ahmad; 3 Farhad M. Abdulkarim Barzinji

Abdulkarim Barzinji, Farhad (1)

(1) Head of the Department, Microbiology Department, Microgene Diagnostic Centre, KISSR & University of Sulaimaniya, Iraq.

Abstract:

Single homo- and hetero-somatic nucleotide transversion mutation in 3 patients (2 males and 1 Female), all of which are members of one family, have been identified at the nucleotide residue C9911G. The mutation leads to amino acid substitution of the aromatic "Phenylalanine" to nonpolar hydrophobic "Leucine" at the N-terminus of the Mt-CO3 protein. Therefore, the present report associates a new mitochondrial amino acid substitution "Phe – Leu" at position 234 with Optical Neuropathy, since no other common mutations have been identified either in the chromosomal DNA or in the Mitochondrial DNA. In addition, a further novel heterosomal deletion mutation has been identified in one of the 3 patients in addition to F234L mutation. The deletion is a stretch of 37 nucleotides starting from nucleotide residue T1207 and terminating at nucleotide residue T1245. In the secondary structure of Human mt-12S rRNA, the deletion in the "3' major domain" is that it encompasses the entire helix 31 (h31). However, we assume that the deletion mutation may have a detrimental effect on the function of Mito-Ribosome due to the configuration in the secondary structure of the mt- 12s rRNA!

Reference:

Vasoprotective Effect of Metformin in Human Umbilical Vein Endothelial Cells through Overexpression of Vascular Endothelial Growth Factor Receptors 1/2 and Their Downstream Signaling under Hyperglycemia and Chemical Hypoxia

Bakhashab, Sherin (1), Ahmed, Farid (2), Schulten, Hans-Juergen (3), Ahmed, Fahad W (4), Glanville, Michael (5), Alqahtani, Mohammed H (6), Weaver, Jolanta (7)

(1) Assistant Professor, Biochemistry, King Abdulaziz University, Saudi Arabia.
(2) Assistant Professor, Centre of Excellence in Genomic Medicine Research, King Abdulaziz University, Saudi Arabia.
(3) , , King Abdulaziz University, Saudi Arabia.
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(5) Assistant Professor, Institute of Cellular Medicine, Newcastle University, United Kingdom.
(6) Professor, Centre of Excellence in Genomic Medicine Research, GenaTi, Saudi Arabia.
(7) Senior Lecturer, Institute of Cellular Medicine, Newcastle University, United Kingdom.

Abstract:

Background: Cardiovascular disease is the leading cause of morbidity and mortality worldwide, particularly in diabetes mellitus. Metformin is the first therapy to provide cardioprotection in type 2 diabetic patients and non-diabetic animals. However, the underlying mechanism behind its action is unknown. Previously, we have shown that metformin improves angiogenesis via affecting expression of growth factors and angiogenic inhibitors in CD34+ cells under hyperglycemia-hypoxia, mimicking acute myocardial infarction in diabetes. Now, we have studied the direct effect of the physiological dose of metformin on human umbilical vein endothelial cells (HUVEC) under in-vitro conditions mimicking hypoxia and short term hyperglycemia. Hypothesis: Metformin exerts its vasculoprotective effect on endothelial cells via enhancing the expression of angiogenic genes. Methods: HUVEC were studied by scratch test for migration and by Annexin V APC staining assay for apoptosis after being cultured in euglycemia (5.5 mmol/l) or hyperglycemia (16.5 mmol/l) for 24 h and then exposed to chemical hypoxia by cobalt chloride (CoCl2) up to 1, 3, or 12 h in the presence or absence of metformin (0.01 mmol/l). Subsequently, HUVEC was assayed by whole transcript microarrays using Human Gene 1.0 ST arrays in three biological replicates for each condition. Raw data files were imported into Partek Genomic Suite and normalized using RMA. Genes of interest were confirmed by quantitative real-time PCR, ELISA and western blots to detect the molecular changes underlying the effect of metformin. Results: At physiological doses, metformin promoted HUVEC migration and inhibited apoptosis via: upregulation of vascular endothelial growth factor (VEGF) receptors (VEGFR1/R2), fatty acid binding protein 4, ERK/mitogen-activated protein kinase signaling, chemokine ligand 8, lymphocyte antigen 96, Rho kinase 1, matrix metalloproteinase 16 and tissue factor inhibitor-2 under hyperglycemia combined with chemical hypoxia. Conclusion: Metformin’s effect in hyperglycemia-hypoxia mediated by direct effect on VEGFR1/R2 leading to dual effect on activation of cell migration through MMP16 and ROCK1 upregulation, in addition to inhibition of apoptosis by increase in phospho-ERK1/2 and FABP4, components of VEGF signaling cascades.

Reference:

Role of microRNA in the pathogenesis of pediatric Acute Lymphocytic Leukemia

El-maadawy, Eman Anwr (1)

(1) PhD student, Molecular biology, GEBRI, Egypt.

Abstract:
Role of microRNA in the pathogenesis of pediatric Acute Lymphocytic Leukemia Eman A. El-maadawy1, Rania M. Bakry2, Mohamed Mousa3, Soby Hasab-El-Naby4, Roba M. Talaat1 1Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City University, Egypt 2South Egypt Cancer Institute, Assiute University 3Clinical Pathology Department, Faculty of Science, Ain-Shams University, Cairo, Egypt 4Zoology Department, Faculty of Science, Menoufiya University, Menoufiya, Egypt Abstract Background: MicroRNAs (miRNAs) are a novel class of small, non-coding RNAs that regulate gene expression at the post-transcriptional level. Abnormal expression of miRNA has been recorded to associate with various types of disease, including cancer. Acute Lymphocytic Leukemia (ALL), a malignancy of B or T lymphoblasts, is the most common form of pediatric malignancy. miRNAs play a significant role in the pathogenesis and progression of acute leukemia and are increasingly recognized to be promising diagnostic and therapeutic targets. Materials and Methods: This work is designed to investigate the clinical significance of miR-21, miR-24, miR-26, miR-148a, miR-155 and miR-133b expression in a group of 43 pediatric ALL patients compared to 42 healthy controls. The expressions of miRNAs were determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). The expression levels of miRNA differed between the two groups. Results: Our results pointed that miR-21, miR-148a, miR-133b and miR-24 and were found to be significantly (p<0.05, p<0.01, p<0.05, p<0.05; respectively) up-regulated in ALL patients compared to controls. On the other hand, miR-155 was found to be significantly (p<0.05) down-regulated in ALL group compared to control group. There was no statistical significance in miR-26a expression level in both groups. ROC analysis showed a cutoff value of 2.064 as the sensitivity of 100% and specificity of 72% for miR-24. In miR-148a, a cutoff value of 1.914, as the sensitivity of 86 % and specificity of 72%. Conclusion: Our data shed some light on the fundamental role of miR-21, miR-155, miR-148a, miR-133b and miR-24, expression in children with ALL, and their great potential value as new novel noninvasive biomarkers for ALL detection. miR-24 and miR-148a upregulation represent an unfavorable prognostic marker in Childhood ALL. Further investigations may reveal the function of these microRNAs and may provide potential targets for novel therapeutic strategies.
Expression Analysis of Pro-Survival Pathways mRNA markers in Basal-like Breast Cancer Subtype

Jaafar, Rola (1)

(1) Senior Research Associate, Surgery, American University Of Beirut, Lebanon.

Abstract:

BC heterogeneity and the inconsistency in clinical response to treatment have led to wide interest in understanding the molecular mechanisms of the different BC phenotypes. Currently, ER, PR and HER-2 are the most widely used markers for BC classification and implement the choice of treatment. Also, gene expression profiling has been widely used to understand the molecular mechanisms involved in disease progression (Kwei et al., 2010), metastasis (Ellsworth et al., 2008), drug metabolism and drug resistance (Normanno et al., 2005; Brennan et al., 2005). In this study, proteins essential for the induction of survival pathways were identified and their relative messenger ribonucleic acid (mRNA) expression was analyzed among different (BC) patient samples obtained from online GEO NCBI database. This is the first study that comprises expression analyses of different isoforms of mRNA expression of genes important in pro-survival pathways regulation, to understand basal-like BC tumorigenesis, and determine main proteins involved in BC prognosis and identify potential therapy targets. The pathways investigated included the PI3K-Akt, mTOR, JAK/STAT, HSR, UPR and NF-κB. Expression of main genes involved in these pathways was compared between the BC subtypes; in addition, their impact on patient prognosis defined by tumor size, grade and LN involvement in addition to patient survival was analyzed. Among the pro-survival pathways analyzed, the NF-κB and to a lesser extent the HSR pathway related genes were observed to be associated with the basal-like BC phenotype, while the AKT and mTOR pathway related genes expression were more associated with the HER-2 BC subtype, displaying worse prognosis. Patient database analyses showed a significant association of NF-κB related genes mRNA expression with basal-like BC, and a negative impact on prognosis and adverse-event free survival. In addition, NF-κB showed a significant positive correlation with MKI-67 expression, which suggests that NF-κB enhances the proliferation rate in basal-like BC. Besides, positive correlation between mRNA expression of NF-κB genes and TP53 was obtained. Genes’ expression and impact on BC prognosis are summarized in the figure below.

Attachment:

The effect of neoadjuvant therapy on the local immune signature in patients with pancreatic cancer.

Mustafa, Dana Adel (1)

(1) Assistant Professor & manager of the Tumor Immuno-Pathology Laboratory, Pathology, Erasmus University Medical Center, Netherlands.

Abstract:

D. Latifi1, D. A. Mustafa2, M. Suker1, C.H.J. van Eijck1 1Dept. of Surgery, Erasmus Medical Center, Rotterdam 2Dept. of Pathology, Neuro-PANC Laboratory, Erasmus Medical Center, Rotterdam. Background: Tumour-infiltrating lymphocytes (TILs) are predictive for response to neoadjuvant chemotherapy in different types of cancer. In pancreatic cancer high tumor infiltration of CD8+ and CD4+ T lymphocytes and low infiltration of T regulatory cells is associated with favorable clinical outcome. However, pancreatic cancer is often characterized by lack of CD8+ T-cell infiltration and by the presence of immunosuppressive myeloid cell populations. It is known that radiotherapy and several forms of chemotherapy can activate the immune system by inducing immune stimulating signals that can increase T cell trafficking to the tumor. The aim of this study is to investigate the effect of the neoadjuvant therapy on the immune profile of the local tumor in pancreatic cancer patients compared to chemo-radiotherapy naïve tumors. Method: Formalin-Fixed Paraffin Embedded (FFPE) tissue samples from resected pancreatic cancer patients in Erasmus Medical Center were used. Gene expression profiling was performed using the nCounter® PanCancer Immune Profiling panel of NanoString technology. The technique enables the comprehensive multiplex gene expression analysis of 770 genes, presenting 24 different cell types of the immune system. Immunohistochemistry was used for the validation of data. Results/discussion: The characteristics of the local immune signature and response in pancreatic cancer will be presented and discussed.

Attachment:

D151Y beta galactosidase mutant causing GM1-Gangliosidosis is rescuable by chemical chaperones and low temperature

Emad, Feda (1), Ali , Bassam (2), AlGazali, Lihadh (3), Al Jasmi, Fatma (4), Ghattas, Mohammad (5), Al Sorakh, Mohammad (6)

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(2) Professor of Molecular and Genetic Medicine, Department of Pathology, College of Medicine d Health Sciences, UAE University, United Arab Emirates.

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(5) Associate Professor , Department of Pharmacy, Al Ain University of Science and Technology, United Arab Emirates.

(6) Assistant Professor, Department of Pharmacy, Al Ain University of Science and Technology, United Arab Emirates.

Abstract:

Background. Missense mutations located outside the enzyme active site might affect protein folding, assembly or stability properties leading to its quantitative loss in lysosomes either due to protein retention and mislocalization to the endoplasmic reticulum (ER) or premature degradation via the ER-associated protein degradation (ERAD) machinery. Lysosomal storage disorders (LSDs) are a heterogeneous subgroup of more than 60 rare inborn inherited metabolic disorders that are usually caused by deficiencies in specific lysosomal enzymes due to genetic defects in their coding genes. GM1-gangliosidosis (OMIM# 230500) is a severe neurodegenerative LSD caused by genetic defects in the GLB1 gene expressing ?-Galactosidase (?-Gal) enzyme resulting in residual enzymatic activity loss in affected cells. The disease has a prevalence of 1 in 100,000–200,000 live births worldwide which is almost 4 times higher in the UAE. A total of 211 mutations have been reported worldwide in the GLB1 gene of which 115 are missense/nonsense mutations. Previous studies have showed that many missense mutations associated with GM1-gangliosidosis affect ?-Gal enzyme three-dimensional structure and confirmation resulting in ER retention and mislocalization. Small molecular weight compounds such as pharmacological chaperones (PCs) and proteostasis regulators (PRs) have shown promising results for overcoming some of these defects in multiple LSDs. These compounds are thought to enhance lysosomal enzyme activity by specific binding to the mutated enzyme or manipulating different proteostasis pathways promoting protein stability, folding, and trafficking and thus enhancing the enzymatic activity of mutated protein in lysosomes and restoring some of its biological function. Aim. Elucidate the molecular and cellular consequences of the GM1-gangliosidosis D151Y missense mutation and evaluate its potentiality to be rescued via chemical chaperones. Methods. Skin biopsy has been collected from an Emirati child with clinical phenotype of GM1-Gangliosidosis and positive family history of the disease; two siblings died with same disease. Fibroblast cells were derived from the skin biopsy via collagenase digestion for further analysis. Mutation analysis of GLB1 by Sanger Sequencing confirmed the presence of a homozygous c.451G>T missense mutation. Loss of enzymatic activity was confirmed through a florescence based ?-Gal activity assay using 4-MU tagged substrate. The D151Y mutated enzyme was created by the Residue Scan module in MOE using the wild type ?-Gal PDB model (ID: 3WF1) and protein stability was confirmed through a florescence based ?-Gal activity assay using 4-MU tagged substrate. The D151Y mutated enzyme was analyzed via running molecular dynamic simulations for 200 ns. D151Y ?-Gal enzymatic processing and trafficking were evaluated via western blot and immunoflorescence analyses. For rescue analysis, patient and control fibroblast cells were treated with increasing glycerol concentrations (0-8%) under normal and low culture temperature. D151Y ?-Gal rescue was evaluated via western blot, immunoflorescence, and enzymatic activity. Statistics were conducted using student t-test. Results. An Emirati child from a consanguineous marriage was clinically diagnosed with type I GM1-gangliosidosis. Whole exome sequencing revealed the previously reported c.451G>T missense mutation at the GLB1 gene which was confirmed by Sanger Sequencing. There was almost complete loss of ?-Gal residual activity in patient’s fibroblast cells with 6 nmol/hr/mg (0.08% of normal control). Computational studies showed that the underlying amino acid change significantly affects the overall 3D structure of the enzyme; hence, the mutated ?-Gal is less stable as indicated by the stability report. D151Y ?-Gal has impaired processing and maturation as only ~11% of ?-Gal precursor in patient fibroblast cells was converted to its mature form compared to ~58% in normal control. In patient fibroblast cells, ?-Gal via immunoflorescence staining was colocalized with calnexin (ER marker) and did not show any lysosomal pattern staining as detected in normal control cells indicating its retention in the ER because of the underlying defect. Glycerol and low temperature enhanced mutated ?-Gal maturation, trafficking, and residual activity. D151Y ?-Gal processing and maturation were enhanced by glycerol in a concentration dependent manner with an increase in the maturation level up to ~35% of normal control while low temperature showed up to ~55% enhancement. Via immunoflorescence, mutated ?-Gal could reach the lysosomes at various degrees under the same conditions which was highest under low culture temperature. Glycerol and low temperature
effect on enzymatic activity was equivalent to its enhancement on protein processing. Similarly, glycerol enhanced enzymatic activity in a concentration dependent manner reaching up to 2.3 folds increase of that in untreated cells while at low temperature enzymatic activity reached up to 7 folds increase compared to untreated patient sample. Conclusion. D151Y mutated β-Gal has impaired protein maturation, trafficking, and low enzymatic activity which were significantly enhanced with glycerol treatment and low culture temperature. D151Y β-Gal potential for rescue is an indication that the underlying mutation is most likely affecting the enzyme folding properties rather than its enzymatic function and the loss of residual activity is due to its quantitative loss in lysosomes. These findings raise the possibility of using pharmaceutical chaperones and proteostasis regulatory compounds as possible personalized treatments for this type of mutation.

Reference:

The Synthesis of Dehydrozingeron and getting other analogues by modifying DHZ structure and compare their biological activities

Ashraf Moawad, Mohammed (1)

(1) B.Ph , Pharmacy, Oman Medical College , Oman.

Abstract:

Introduction Dehydrozingeron is one of the most effective constituents in ginger oil extract which is having different biological activities such as antioxidant, hepato-protectant, GABA aminotransferase inhibitor, anti-inflammatory, interleukin antagonist, platelet adhesion inhibitor, 5HT release stimulant, JAK2 inhibitor, MMP9 inhibitor, TNF inhibitor and anti-hypercholesterolemic properties. Methodology In our research, we synthesized DHZ in the lab by dissolving vanillin with acetone in room temperature and then acidification by hydrochloric acid 10% and then checking the purity by melting point which was 130 equal to standard temperature. Also, we modified this structure by using chemsketch software into twenty-one analogues and then checked their biological activities mentioned above and started the comparison between them. And to validate our biological activities values, we compare all analogues as well as lead compound with a drug in the market which has same mechanisms of action, surprisingly, we found fluctuations between values for analogues and drugs, some of them prove that analogues have more efficacy than drugs and the other was likewise. Results & discussion Actually, antioxidant activity was the first property to check. And, we found that analogue D as well as analogue C have higher free scavenger radical than glutathione and vitamin C. Also, analogue I has shown anti-inflammatory value which is equal 99.8% of ibuprofen but it has 236% antioxidant activity which means it’s safer than ibuprofen. Moreover, analogue C has more selectivity on JAK2 expression inhibitor than tofacitinib and ruxolitinib. In fact, scientists now are looking for any mechanisms which inhibit MMP9 expression to treat many autoimmune diseases such as two drugs under pre-clinical trial batimastat and marimastat but we found that our analogue C has more selectivity and efficacy on MMP9. Unfortunately, TNF expression inhibitors their structures are not available because most of them are monoclonal anti bodies, but we find that analogue C has 90.7% efficacy on TNF expression inhibition. Furthermore, we found that analogue C is 1.5 times more effective than nicotinic acid on anti-hyperlipidemic activity. The study shows that analogue C is effective as antioxidant, JAK 2 expression inhibitor, MMP9 expression inhibitor, TNF expression inhibitor and anti-hyperlipidemic activities. Also, analogue I is 99.8% anti-inflammatory compare to ibuprofen as well as it’s 236% safer than ibuprofen.

Reference:


Attachment:

Discussion:
http://membs.org/membs/uploads/congress_speaker_files/1527775728Discussion.docx

Pass values Vs Biological activities 1:
http://membs.org/membs/uploads/congress_speaker_files/1527775728-Pass online last 1.docx

Pass values Vs Biological activities 2:
http://membs.org/membs/uploads/congress_speaker_files/15277757284-pass online last 2.docx
Across-sectional study of urinary tract infection with ESBL-producing E. coli in tertiary Jordanian hospitals: Prevalence, genotyping and risk factors

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(1) Master Student, pharmaceutical microbiology, University of Jordan, Jordan.
(2) , , The University of Jordan- The School of Pharmacy, Jordan.
(3) Dean of Pharmacy school, Biopharmaceutics and Clinical Pharmacy, The University of Jordan- The School of Pharmacy, Jordan.
(4) Associate Professor at internal medicine, Internal Medicine, The University of Jordan- The School of Pharmacy, Jordan.

Abstract:

Background: Extended spectrum β-lactamases (ESBLs) are one of the most problematic groups of β-lactamases in clinical practice. The aims of this study were to determine the prevalence, phenotypes, and genotypes of ESBL-producing Escherichia coli (E. coli) among patients with urinary tract infection and to identify the associated risk factors. Methods: A cross-sectional study was conducted at the Jordan University Hospital (JUH) and Islamic Hospital (IH) in Amman, Jordan between June and October, 2016. One hundred twenty one E. coli isolates from hospitalized patients ( > 3 days after hospital admission) with urinary tract infection were phenotypically assessed for ESBL production using the double disc diffusion test. Positive isolates to ESBL production were further genotyped using multiplex PCR. A nested case-control study was used to determine the independent risk factors. Cases were identified as hospitalized patients with symptoms related to UTIs and a positive urine culture for ESBL-producing E. coli, while controls were patients with UTIs due to none ESBL-producing E. coli. Cases of ESBL-producing E. coli were matched in a 1:1 ratio to control of none ESBL-producing E. coli according to age, gender, and settings. Results: ESBL-producing E. coli were found in 75/121 (62%) isolates. Molecular genotyping demonstrated that CTX-M group1(42.7%) predominated followed by combination of SHV and CTX-M group1(20%). Univariate analysis showed that hospitalization in the previous 6 months, history of out clinic visits, using urinary catheter and recurrent symptomatic urinary tract infections were associated with UTI infections due to ESBL-producingE. coli. In the regression model, previous hospitalization and use of urinary catheter were identified as independent risk factors for ESBL-producing E coli infections. Conclusion: Our findings demonstrate that the prevalence of ESBL-producing E. coli is high but it is still in concordance to other studies from developing countries. Additionally, CTX-M group1 has emerged as the predominant ESBL produced by E. coli, which is consistent with reported results throughout the world. Independent risk factors to UTI infections due to ESBL-producing E. coli include previous hospitalization and use of urinary catheter.
Molecular Mechanism of Antiemetic Effect of Menthol

Al Kury, Lina (1)

(1) Assistant Professor, Health Sciences, Zayed University, College of Natural and Health Sciences, United Arab Emirates.

Abstract:

Menthol, a naturally occurring cyclic monoterpene alcohol, is a major active ingredient of the peppermint plant. Since antiquity, peppermint has been used widely for various medicinal purposes ranging from management of musculoskeletal pain and the common cold to the treatment of gastrointestinal disorders such as nausea and vomiting. In addition, menthol is known for its cooling, antioxidant, anti-inflammatory and antiseptic effects. Recently, there has been renewed awareness in comprehending the biological and pharmacological effects of menthol. However, the cellular and molecular targets mediating the beneficial effects of menthol are still not well-understood. Evidence show that menthol can significantly influence the functional characteristics of a number of ligand and voltage-gated ion channels. Human serotonin type-3 (5-HT3) receptors are proposed to play an important role in motility of gastrointestinal system, in nociception, neurodevelopment, and in psychiatric disorders such depression. The actions of menthol on the function of human 5-HT3A receptors expressed in Xenopus laevis oocytes were tested. 5-HT-evoked inward currents were recorded using two-electrode voltage clamp. Currents were reversibly inhibited by menthol in a concentration-dependent manner with an IC50 value of 163 ?M. The inhibitory effect of menthol developed gradually, reached a steady-state level within 10-15 minutes and did not involve G-proteins. The effects of menthol on 5-HT3 receptor-mediated currents were not stereoselective as (-), (+), and racemic menthol inhibited 5-HT3 currents to the same extent. The maximum inhibition induced by menthol was not surmounted by increasing concentrations of 5-HT. Moreover, specific binding of the 5-HT3 antagonist [3H]GR65630 was not changed in the presence of menthol. The results of this study indicated that, at pharmacologically relevant concentrations, menthol acts as a negative allosteric modulator of 5-HT3 receptors. This effect may play an important role in the treatment of chemotherapy-induced nausea and vomiting.

Reference:


Attachment:

Abstract_menthol:
Pomegranate seed oil improve insulin sensitivity in high-fat and high-sucrose diet-induced obesity mice model

HARZALLAH, ARIJ (1), HARZALLAH, ARIJ (2)

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(2) Researcher, Research Laboratory ‘Nutrition – Functional Food & Vascular Health’, Faculty of Medicine, University of Monastir, Monastir, Tunisia, Researcher, Tunisia.

Abstract:
The excessive intake of high-fat and high-sucrose (HF/HS) diets is one of the primary causes of obesity and related metabolic disorders. Epidemiological and biochemical studies suggest that a high dietary intake of fructose is an important causative factor in the development of the metabolic syndrome commonly characterized by a perturbation of glucose metabolism, insulin resistance induction and dyslipidaemia in humans and in animal models. Alternative medicines and natural therapies offer a potential treatment for type-2 diabetes but without the prominent side effects of some synthetic drugs. Many traditional plant treatments for diabetes are used throughout the world. Pomegranate extracts have been reported to have several beneficial effects on hyperglycaemia and obesity-related inflammation. These beneficial effects may be due to its composition containing 72% of the conjugated linolenic fatty acid and punicic acid. In our study, the effect and the action mechanism of pomegranate seed oil (PSO) is described in a nutritional model of inflammation and insulin resistance. After four weeks, treatment with PSO reduce fasting blood glucose. Pomegranate seed oil is shown also to decrease plasma levels of the pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-α) and interleukin-6. This beneficial effect may be a result of an improvement of insulin sensitivity shown during the insulin tolerance test and which is similar to the response of the rosiglitazone-treated group with a significant increase in IL-10 and interferon-gamma (IFN-γ). Beyond its beneficial effects on inflammation related to obesity, PSO could exhibit a potential insulin sensitizer property mediated through its anti-inflammatory properties if used for long period.

Reference:
Effect of Gold Nanosphere in Treatment of Collagen Induced Arthritis (CIA) in Wister Rat

talaat, roba (1), Abdel hakiem, Neha (2), Abo El atta, amira (3), alkilany, Alaaldin (4), Alkawareek:, Mahmoud (5), samaka, Rehab (6)

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(2) PhD Student and researcher, Molecular Biology Lab, GEBRI, University of Sadat City, Egypt.
(3) phd student, Molecular biology, GEBRI, Sadat City University, Egypt.
(4) Associate Professor in Nanoscience and Pharmaceutics, Department of Pharmaceutics and Pharmaceutical Technology, University of Jordan, Jordan.
(5) Assistant Professor in Pharmaceutical microbiology, Department of Pharmaceutics and Pharmaceutical Technology, University of Jordan, Jordan.
(6) professor of pathology, pathology department, faculty of medicine, menofia unvrisity, Egypt.

Abstract:

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease with no apparent cure. Despite the presence of different classes of medications that have greatly improved the quality of life of patients, several drawbacks are present. Nanotechnology has recently stood out as a promising field for the treatment and diagnosis of a variety of diseases including RA. Gold nanoparticles (GNPs) are particularly hopeful due to its ease of synthesis in various shapes and sizes beside availability for conjugation with peptides and proteins, which can target the GNPs to specific interaction partners. However, role of GNP in the treatment of autoimmune diseases like RA remains vague. Therefore, the aim of this work was to study the effect of different sizes of gold nanosphere on collagen induced arthritis (CIA) in rat and their distribution in different organs. Materials and Methods: This study was conducted on sixty female Wistar rats divided into 6 groups (10 animals each): Group (1) Normal untreated rats (negative controls), Group (2) was CIA rats without treatment (positive control group), Groups 3, 4 and 5 were rats treated with [small size GNPs (5nm), medium size GNPs (25nm) and large size GNPs (75nm), respectively]. The ankle joints were injected intraarticular with 6.5 µg of pegelated (PEG-GNP) suspended in 65µl of ultra-pure water on day 11 after induction of CIA. Group (6) was rats received 4 intraperitoneal injections of methotrexate (MTX) (1 mg/kg) on days 11, 17, 24, 31 after induction of CIA. At the end of the experiment (day 35), Clinical and radiographic assessment were done. Organ weight and histological changes in the joints and internal organs were also investigated. The presence of gold was measured quantitatively with inductively coupled plasma mass spectrometry (ICP-MS). Results: Our results reported that Radiographs showed a slight change in joint space width, degree of bony destruction, and soft tissue swelling after nanoparticle treatment. GNPs were deposited in all the examined organs (liver, lung, kidney, spleen, brain and heart) and also showed that the accumulation was size dependent with the smallest size (5nm) nanoparticles demonstrated the most distributed in comparison with medium and large ones. Histopathological results revealed that intraarticular administration of GNPS (25nm) reduced the inflammatory cell infiltration, joint swelling, cartilage erosion, bone destruction and development of polyarthritis. Conclusion: Our results suggest that nano-gold has size dependent effect on treatment of RA. Nanosphere with 25 nm has a good therapeutic effect for ameliorating RA. Hence, GNPs could be used to establish a new class of anti-arthritic drug that’s more effective and has fewer side effects.
Genetic Regulation of Cytokine Secretion in Egyptian Patients with Hepatitis B Viral Infection

ABDELKHALEK, MOHAMED (1), Talaat, Roba (2)

(1) MSc Student, Molecular biology, GEBRI, Egypt.
(2) Ass. Prof. Molecular Immunology, Molecular Biology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt, Egypt.

Abstract:

Background: To our knowledge, controlling cytokine secretion profile could be reflected by genetic basis such as cytokine gene polymorphism and expression of certain miRNA. Through this work, we intended to have a good documentation about the most prominent polymorphisms in different pro/anti-inflammatory cytokine (IL-6, TNF-?, IL-10, TGB-? and IFN-?) genes associated with chronic HBV infection and to examine the expression of various miRNA that have a role in their expression. We hypothesized that this could affect the pathogenesis of HBV. Materials and Methods: Polymorphisms of the IL-6 (-174G/C, -572G/C, -597G/A), TNF-? (?863C/A, ?308G/A), IL-10 (-1082 G/A, -819 C/T), TGF-?1 (+869T/C, +915G/C), IFN-? (+874A/T, +2109A/G) were analyzed by Polymerase chain reaction sequence specific primer (PCR-SSP) while TNF-? (?376G/A, ?857C/T and +489G/A) and TGF-?1 (-800G/A, -509C/T, and + 788C/T) were analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) in HBV patients and normal healthy controls. Quantitative real time PCR (qRT-PCR) using miScript RNA/RT/SYBR Green kit for the detection of miR-10, miR-17, miR-21, miR-24, miR-26, miR-122, miR-125, miR-145, miR-146, miR-148a, miR-155 and miR-221 was carried out PCR Kit. The effect of genetic variability (either as polymorphism or expression of certain miRNA) on the cytokine secretion profile was examined by measuring the serum levels of tested cytokines in using Enzyme-linked immunosorbent assay (ELISA). Results: IL-6 (-174G/C, -572G/C, -597G/A), TNF-? (-863C/A, -308G/A, -509C/T, and + 788C/T) were significantly increased in HBV infected patients as compared with healthy subjects. On the other hand, miR-10, miR-17, miR-21, miR-24, miR-26, miR-122, miR-125, miR-145, miR-146, miR-148a, miR-155 and miR-221 were significantly decreased. A significant reduction in IL-6, TNF-? and IL-10 secretion level levels were demonstrated coincides with a significant elevation in IFN-? and TGF-? in HBV patients in relation to normal controls. Changes in cytokine secretion level observed in HBV patients were irrelevant to any of tested genotypes. Elevation of IL-10 was positively correlated with elevation of mir-21, mir-24, mir-26, mir-122 and mir-146. A negative correlation was recorded between reduction of IL-6 and elevation of mir-26, and mir-146. Reduction of TNF-? was positively correlated with reduction in mir-17 and mir-221 and negatively correlated with elevation in mir-122 and mir-146. Conclusion: Although our results stressed on the importance of certain cytokine SNPs on HBV susceptibility, the real effect of this genetic mutation on cytokine production was not detected. Cytokine-associated miRNAs such as mir-21, mir-24, mir-26, mir-122 and mir-146a, are frequently detected in HBV infection; however, their functions are still not fully clear. Thus, the discovery of certain genetic predisposition with regulatory capacity of cytokine secretion response during HBV infection will open a novel track for the diagnosis and prevention of HBV through effectively modulating inflammatory/anti-inflammatory cytokines.
Actin in gene regulation and genome organization during development

Percipalle, Piergiorgio (1)

(1) Principal Investigator, Science Division, Biology Program, New York University Abu Dhabi, United Arab Emirates.

Abstract:

During development and differentiation, waves of genetic reprogramming ensure that cells acquire specific identities. In eukaryotic cells, this is partly achieved by regulated changes in genome organization, a mechanism that primarily relies on the spatial distribution of chromatin and epigenetic modifications in response to environmental cues. Long-range movement of gene-rich chromatin loops and formation of DNA loops within the nuclear interior ensure establishment of neighborhoods of both gene expression and gene silencing. This consolidation of the functional architecture of the cell nucleus leads to sustained expression or silencing of genes involved in development and differentiation. The rules that control the dynamic properties of chromatin, however, have yet to be determined. Although heterochromatin is known to remain segregated at the nuclear lamina whereas gene-rich euchromatin is preferentially maintained at the nuclear interior for active transcription of gene programs, the main players responsible for this specific organization have not been identified. Another key challenge is to find out whether genes involved in the same cellular functions are hosted within the above mentioned nuclear neighborhoods for sustained expression or silencing over time. Understanding these questions will shed light on the mechanisms underlying cellular differentiation when cells acquire specific identities. Emerging evidence indicates that cytoskeletal proteins play important roles in the cell nucleus. Actin and actin-based molecular motors such as myosin have been shown to regulate gene activity, chromatin structure and function. More recently, we provided evidence supporting a direct involvement for both nuclear actin and myosin in the organization of the mammalian genome, activating and repressing entire gene programs during cellular development and differentiation. Here, I will review these recent studies and discuss how these results support the importance of actin-based mechanisms are likely to impact differentiation and, consequently, the acquisition of cellular identity.

Reference:

Association of microRNA expression with the clinicopathologic characteristics of ependymoma

Al-Hussaini, Maysa (1), Ahram, Mamoun (2), Amarin, Justin (3), Suradi, Haya H. (4), Abdelhamid, Sultan S. (5), Bawadi, Randa (6), Makhamreh, Mona (7)

(1) Consultant Histopathologist/Neuropathologist, Department of Pathology and Laboratory Medicine, King Hussein Cancer Center, Jordan.
(2) Associate Professor, Physiology and Biochemistry, The University of Jordan-School of Medicine, Jordan.
(3) , , The University of Jordan, Jordan.
(4) Undergraduate Student, School of Medicine, The University of Jordan, Jordan.
(5) Undergraduate Student, School of Medicine, The University of Jordan, Jordan.
(6) Research scientist, Physiology and Biochemistry, The University of Jordan-School of Medicine, Jordan.
(7) Undergraduate Student, Undergraduate Student, The University of Jordan-School of Medicine, Jordan.

Abstract:

The molecular classification of disease, including cancer, may greatly benefit the provision of healthcare to patients. MicroRNAs (miRNAs) are short, non-protein-coding RNA molecules that regulate gene expression by modulating the stability and transcriptional use of RNA transcripts. Depending on their differential expression, miRNAs may act indirectly as oncogenes or tumor suppressor genes in cancer cells. Therefore, they may be used to classify cancers such as ependymoma. Ependymoma is a central nervous system tumor that occurs in the intracranium or the spine of pediatric and adult patients. The prognosis of ependymoma is influenced by patient age, anatomic location, extent of surgical resection, and histologic grade. The aim of our study is to assess the association of miRNA expression with the clinicopathologic characteristics of ependymoma. Twenty-two ependymal tumor samples with annotated clinicopathologic data were retrieved from the King Hussein Cancer Center. Using PCR arrays, the expression profile of 84 stem cell-specific miRNAs was evaluated. Six grouping variables—namely, anatomic site, recurrence status, histologic grade, the Ki-67 proliferation index, cyclin D1 immunoreactivity, and nestin immunoreactivity—were used to generate differential miRNA expression profiles. Real-time PCR data were interpreted using the \( \Delta \Delta CT \) method of relative quantification. Nineteen tumor samples were included in the final analysis. Nestin immunoreactivity did not significantly discriminate the expression of any miRNA under study. Analysis of the other grouping variables revealed a total of 19 differentially expressed miRNAs. Seven markers (miR-106b-5p, miR-10a-5p, miR-17-3p, miR-19a-3p, miR-196b-5p, miR-323a-3p, and miR-93-5p) were location-specific. Markers in infratentorial tumors were generally overexpressed compared to supratentorial and spinal tumors. However, markers in supratentorial tumors were underexpressed compared to spinal tumors. All six recurrence markers (miR-192-5p, miR-22-3p, miR-222-3p, miR-326, miR-371a-5p, and miR-520g-3p) were overexpressed in recurrent cases compared to nonrecurrent cases. Three markers (miR-122-5, miR-134-5p, and miR-520e) were underexpressed in high-grade lesions compared to low-grade lesions. In addition, two markers each were underexpressed in samples reactive to Ki-67 (miR-24-2-5p and miR-520e) and cyclin D1 (miR-103a-3p and miR-24-3p) compared to nonreactive samples. In conclusion, molecular alterations in ependymoma involve miRNAs. Variable levels of evidence support the candidacy of markers identified herein. Putative target proteins of reproducible miRNA markers should be investigated in future studies.
Pyrilium compounds in quantitative assays and evaluation of proteolysis

Al-Essa, Mohamed K. (1)

(1) Assist. Professor, Department of Physiology and Biochemistry, The University of Jordan/ Faculty of Medicine, Jordan.

Abstract:

Introduction: Fluorogenic pyrlium (Py) salts are attracting interests by yielding high fluorescence upon reaction with primary amine. They have been applied in a variety of detection and assay techniques [1-8]. Their high reactivity with primary amines in alkaline media can be detected either by following their chromatic or spectral changes [9-11]. Shifts in their characteristics after reaction are having advantage in differentiating between signals generated from free and conjugated dyes [12]. In addition to their potential use in labeling biomolecules, pyrlium salts could have importance in measuring protein concentration with high accuracy in unknown samples after generation of standard curves. Since these compounds are assaying the reactive amine groups in samples, which are increasing by proteolysis, these compounds could also have potential in checking proteolytic activity in samples by measuring concentration of amines before and after storage of protein samples. Methodology: In this study, we have assessed the potential applications of two pyrlium dyes (one is commercially available known as chromeo P503 and the second is sold as a rare product without being characterized for labeling or detection) for use in estimation of amine modified ssDNA, generation of standard concentration curves with known concentrations of bovine serum albumin (BSA) and proteolysis testing by the use of trypsin. Fluorescence intensities (FIs) were measured and for some samples, spectral histograms were recorded by spectrofluorometry techniques. Plots for results were generated by using Microsoft EXCEL 10. Results: Low FIs were detected for the free dyes, while the reaction with amine results in high fluorescence signals. Spectral histogram recordings for the new pyrlium after reaction with amine modified ssDNA (prepared in different concentrations as 5nM, 50nM and 500nM) were presented in figures 1 and 2. It is clearly seen, increased peaks of fluorescence in histograms by increasing concentration of the amine modified ssDNA. In this study, we have used also three types of modified ssDNA. One is having only one amine modification, the second is with three amine modification and the third with nine amine modification at the 5’ ends. It was clearly shown high differences in fluorescence emission by new Py with the three concentrations for each ssDNA, and at high intensity set up recording; the peaks were above the recording limits with the highest concentrations for the second and the third amine modified ssDNA. Chromeo P503 and the new pyrlium compounds were evaluated for generation of standard concentration curves with BSA (Fig. 3). We have worked around detection limits established for Chromeo P503 (known also as Py1) in previous studies [9, 10]. In this part, lower concentration of new Py dye was used than in other assays. The standard concentration curves generated from data were reliable with R2> 0.95. The higher slope and the calculated R2 for the data used could give superior advantages for the new pyrlium in protein assays. Because of the higher sensitivity of the new pyrlium, we have used higher concentration of new pyrlium to generate another standard curve with BSA concentrations <200nM (713.33µg) (Fig. 4). Results are showing high sensitive testing by using New Py and as low as 0.606nM (corresponding to ? 40ng of BSA) were quantified by Shimadzu 5301 PC spectrofluorometer. The high sensitivity of detection of Py compounds was encouraging to check the assay of hydrolysis of proteins in solution by a simple technique. Since hydrolysis of protein results in increasing amine groups, I have measured FIs of BSA in samples containing trypsin. It was clearly shown the difference at 1 hour for samples assayed with Chromeo P503 (Fig. 5). Even more clear differences were observed in samples collected at 5 minutes and assayed with the new pyrlium (Fig. 6). Conclusion: Since we can generate a reliable standard curve for protein assay, Py compounds can have high potential in estimation of protein concentration. The compounds have also potential for estimation of reactive amine concentration, which may help in labeling techniques of biomolecules. Estimation of proteolysis of samples is also possible by the use of Py compounds.

Reference:


**Attachment:**

All Figures:

Final Figures:
Gene Mutation of BRCA 1 & 2 Genes association with Breast & Ovarian Cancer.

Rubi, Ghazala (1)

(1) Director, Central Research and Diagnostic Lab Molecular Genetics Section, Post Graduate Medical Institute Lahore Pakistan, Pakistan.

Abstract:

Abstract: BRCA1 and BRCA2 (Breast Cancer genes 1 and 2) are the best-known genes linked to breast cancer risk. Everyone has these genes, but some people have an inherited mutation in one or both that increases the risk of breast cancer. BRCA 1 gene is a tumor suppressor gene and participates in DNA damage and its repair. BRCA1/2 mutations can be passed to you from either parent and can affect the risk of cancers in both women and men. A person who has a BRCA1/2 mutation is sometimes called a BRCA1/2 carrier. Like other gene mutations, BRCA1/2 mutations are rare in the general.

Findings: The overall mutation rate of BRCA1/2 genes found in 17.9% patients of our local population. Whereas, the mutation rate was more than 40% in all registered patients with inherited breast /ovarian cancer. But further analysis of clinical pathology parameters revealed no significant correlation of BRCA genes mutation with patient’s age, menstrual status and metastasis. Few families were tested and found that breast cancer patients with BRCA genes 1 & 2 mutation are more favorable prognosis. Conclusion: Genetic and molecular testing of BRCA Gene 1&2 designed and developed. A cohort study is undergoing for future findings and clinical implications.
Effect of Social Stress on Circadian Rhythms and Sleep-Wake Cycle

Chaudhury, Dipesh (1)

(1) Assistant Professor, Biology, New York University - Abu Dhabi, United Arab Emirates.

Abstract:

Though it is known that daily rhythms are disrupted in patients suffering from mood disorders, the molecular mechanisms linking aberration in circadian / sleep rhythms and mood disorders is still not well understood. Observations that brain regions associated with mood regulation have robust neural connections, and overlapping molecular pathways, with regions that regulate biological rhythms allow us to investigate the link between these brain regions following expression of depression-like behaviour. We are using a combination of rodent behavioural model of stress together with electrophysiological and molecular approaches to investigate changes in physiological and molecular dynamics between brain regions that encode mood, circadian rhythms and sleep/wake rhythmicity in mice that are resilient (non-depressed) and susceptible (depressed) to social defeat stress.
Premarital Screening: Is it ethical?

Adlan, Abdallah (1), Almutairi, Adel (2)

(1) Chairman, Biomedical Ethics, King Abdullah International Medical Research Center (KAIMRC), Saudi Arabia.
(2) Deputy Chairman, Research office, King Abdullah International Medical Research Center (KAIMRC), Saudi Arabia.

Abstract:
This paper is going to discuss an ethical thorny issue regarding the mandatory premarital screening. The argument is that compulsory premarital screening is more problematic than the elective premarital test. The main argument is going to be around the razing star of new age medicalization and contemporary eugenics. Hospitalization: It is the notion that human issue should be looked at as a medical condition. For example, pregnancy has become looked as condition attracts the full attention of the health caregiver rather than just a natural process that human beings have been doing it for ages even before medicine. The main mindset of health caregiver is to use the cutting edges tools to make people live better. This has made the health caregiver expect of himself to fix the fixable and to prevent the unfixable. This could be acceptable if we are talking about any proficient other than medicine. This is because prevention measure could mean preventing the compromised person to exist. Measure like, birth control, genetic selection, and now mandated premarital tests are geared towards conditional acceptance of new babies. Unconditional love and acceptance seem to be no longer the case. Health caregivers are trying to produce babies who are suitable for health caregiving budget or the health caregiver empathy. The narrow vision of medicalization and the health caregivers ego seems to dominate the discussion. Rarely to see an ethical, legal, cultural voice participating without the nudging to accept. The other major ethical challenge, mandating the test in some context would just give the person the burden of knowledge. The health caregivers assumed that the results will be discussed with a genetic counselor. This is an impossible goal to be achieved with limited resources. People in remote locations and villages will still get to do the test even if they cannot afford to go the nearest big city to get the counselor. This means that they are lifted with a data that they cannot process which leads to so many ethical challenges like stigmatization for example. In conclusion: I think that the notion of mandating premarital test is not fairly ethically discussed. It ought to be fairly upraised and the revisited with a wider lens than the health caregivers’ ones

Reference:
Skin Isolated Mesenchymal Stem Cells Predict Disease Behavior of defected SORCS3, Sortilin Related VPS10 Domain Containing Receptor 3

Al Rafaei, Bahauddeen (1), Alfadhel, Majid (2), Albahkali, Sarah (3), Almuaysib, Amani (4)

(1) Associate Research Scientist, Stem Cells and Regenerative Medicine, KAIMRC- Ministry of National Guard - Health Affairs, Saudi Arabia.
(2) Genetic Consultant, Genetic division, Ministry of National Guard Health Affairs, Saudi Arabia.
(3) Med. Technologist, Stem Cells, KAIMRC- Ministry of National Guard Health Affairs, Saudi Arabia.
(4) Med. Technologist, Stem cells, KAIMRC- Ministry of National Guard Health Affairs, Saudi Arabia.

Abstract:
The sortilin-related VPS10 domain-containing receptor 3 (SORCS3) is type-I receptor transmembrane protein and a member of the vacuolar protein sorting 10 receptor family. Proteins of this family are defined to have a vacuolar protein sorting 10 domain at the N-terminus. They play important roles as a sorting agency within the cells and transport a variety of intracellular proteins between the Golgi apparatus, endosome, lysosome, secretory granules, and plasma membrane. They are also involved in signal transduction. Clinically, they have been implicated in the pathophysiology of multiple sclerosis and Alzheimer disease. Here, we report a link between SORCS3 and global developmental delay. In addition, delayed myelination could be a response of SORCS3 defect. Whole exome sequencing and autozygome analysis showed homozygous missense variant in SORCS3 gene in patient’s sample. The pathogenicity is supported by functional studies of the patient mesenchymal stem cells. Patients’ cells showed less proliferation capability than normal by 60%. In addition, making the same mutation in normal cells revealed a viability defect on them by 40%. This is the first study on human subjects with a SORCS3 gene defect and supports the important role of SORCS3 in the central nervous system.
Lipid profile and apolipoprotein E genotyping in Algerian patients with Alzheimer's disease

Makrelouf, Mohamed (1)

(1) Director of Research Laboratory Biochemistry Genetics, CHU Bab El Oued - Université Alger 1, Algiers, CHU BAB-EL-OUED, Algeria.

Abstract:

BACKGROUND: Cholesterol, an essential component of cell membranes, plays a fundamental role in the development, maintenance and plasticity of neurons. Furthermore, Apolipoprotein E (ApoE), a lipid metabolism protein, plays also an important role in the growth, repair, and maintenance of the CNS. Several studies have reported that hypercholesterolemia promotes the development of Alzheimer's disease (AD), although the mechanism of this relationship is not fully understood. The E4 allele of the APOE gene, a genetic susceptibility factor for Alzheimer disease is strongly suspected, by its hypercholesterolemic effect, to be involved in this mechanism. OBJECTIVE: The aim of this study is to investigate lipid profile parameters and apoE genotypes in AD among Algerian population. MATERIAL AND METHODS: We examined the lipid profile parameters levels of 156 cases of Alzheimer disease and 127 age-matched normal healthy controls. Lipid parameters were performed with colorimetric enzymatic methods on Cobas Integra 400 (Roche Diagnosis). Meanwhile, ApoE genotypes were determined by PCR performed on LightCycler instrument. RESULTS: E4 allele frequency in cases is 6 times greater than that of controls. Blood cholesterol and triglyceride levels are significantly increased in cases, especially in E4 allele carriers. Paradoxically and surprisingly, the HDL levels, although not statistically significant, were higher among cases, corroborating with reduced ApoAI levels. CONCLUSION: Our results have also shown significantly high blood cholesterol and triglycerides levels in Alzheimer Disease, especially those carrying the E4 allele, thus confirming the hypercholesterolemic effect of this allele.
**Abstract:**

The development of massively parallel sequencing and advanced sequencing chemistry has led to the introduction of different sequencing platforms and techniques. This recent emergence of advanced molecular techniques has exponentially improved our ability to explore and understand the cancer genome. Characterisation of somatic mutations acquired by cancer patients is highly applicable to clinical studies, in which some mutations appeared as actionable targets for personalized therapy. Chronic lymphocytic leukaemia (CLL) is a low-grade lymphoma characterised by clinical and biological heterogeneity. The clinical progression ranges from years without requiring treatment to a rapid progression with refractoriness, and none of the conventional treatment options are curative. CLL cases lack disease-defining mutations but they can be broadly classified into two prognostic groups by the immunoglobulin heavy chain variable (IgHV) gene mutational status, where IgHV unmutated status identifies a distinctly worse prognostic group. Recent genome-wide technologies have identified multiple additional recurrent alterations, some of which may have independent prognostic value. The increase number of potential CLL genomic markers may necessitates the simultaneous screening of multiple genes in the clinic. Next Generation Sequencing (NGS) technologies offer significant advantages over other conventional molecular techniques in screening these genes, however, they have not been evaluated sufficiently, nor standardised for clinical implementation. Most CLL whole genome sequencing (WGS) and whole exome sequencing (WES) studies investigating the genetic heterogeneity of CLL have looked only at the coding regions, and data concerning the significance of recurrent mutations in regulatory elements is lacking. The elucidation of CLL genomic complexity and heterogeneity may contribute to our understanding about molecular pathogenesis in CLL, and may subsequently lead to an improved clinical management through specifically designed targeted therapies. Accordingly, we designed in-silico pipeline to functionally annotate somatic variants using biological markers identified by ENCODE ChIP-Seq (chromatin immunoprecipitation sequencing), along with CLL specific ChIP-Seq and ATAC-Seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing). Here we present a comprehensive WGS analysis of somatically acquired mutations from 46 CLL patients, including a systematic comparison of coding and non-coding single-nucleotide variants, recurrent structural variants and copy number, kataege regions and mutational signatures between the two IgHV groups. Functional annotation of WGS data of 46 CLL genomes revealed distinct genomic profiles in the two CLL IgHV subgroups, which may be explained, to some extent, by the prevalence of extracted mutational signatures in the two groups. Whereas the incidence of coding mutations affecting CLL driver genes including TP53, SF3B1, and NOTCH1 was higher in unmutated IgHV patients, the incidence of non-coding mutations affecting promoter regions in IgH locus and genes targeted by SHM (Somatic hypermutation) including BCL5, BCL2, BCL11A, BCL6, and BACH2, was higher in cases with mutated IgHV. Additionally, our analysis identifies recurrent mutations within regulatory elements of important transcription factors required for B-cell development including PAX5, IKZF3, and IKZF1 as well as other genes implicated in cancer including BIRC3. Our findings support the hypothesis that differences in clinical outcome and biological characteristics between the two subgroups might reflect differences in mutation distribution and underlying mutagenic mechanisms. Additionally, we technically compared between the precision of targeted sequencing panel approach (TSP) and WGS for sequencing CLL recurrently mutated genes in the 42/46 CLL patients to evaluate the two techniques for diagnostic service. While WGS offers a high throughput screening of whole genes, TSPs appear to be the most logical approach for replacing Sanger sequencing and improving clinical management of CLL patients in routine care, and a 32 gene CLL panel based on this original panel design has now been introduced into Oxford Molecular Diagnostics Centre diagnostics. Moreover, this study has directly led to establishing a systemic validation protocol to examine targeted gene panels in preparation for clinical diagnostic services and to maintain their precision in the diagnostic service following on from software updates to prevent drift in results. Taken together, we demonstrate that NGS technologies are powerful research tool that can help us to expand our knowledge about the relationship between disease pathogenesis and genetic variations in CLL patients, and can be fully adopted for clinical service.
Current Targeted Therapeutic Strategies for Monogenic Disorders

Abu-Baker, Aida (1)

(1) Research scientist, Department of Neurology and Neurosurgery, McGill University, Canada.

Abstract:

In single-gene diseases, a mutation in just one of these genes is responsible for disease. For many single-gene disorders, the genetic basis is well understood, and the disease-causing gene variants can be identified with genetic testing. However, only a relatively small number of genetic disorders have been amenable to definitive treatment. Major advances in understanding the molecular basis of genetic disorders, however, have brought new hope that improved means of treatment will be developed. Identification of the gene responsible for a disorder leads to elucidation of the dysfunctional cellular and physiological pathways, and may lead to the discovery of new targets for drug therapy. Here, we discuss our current treatment strategies for a muscular dystrophy disease (an example of monogenic disease). We present three experimental therapeutic approaches: pharmacological, RNA replacement, and crispr-cas9 based gene editing. Our scientific findings could ultimately lead to an effective therapy for patients with single-gene diseases.

Reference:


Attachment:
Figure_Single Gene Disease Therapy:
http://membs.org/membs/uploads/congress_speaker_files/1529005401Figure_Single Gene Disease Therapy .pdf
Development of a Robotic 3D Bioprinting System for Printing of Tissues and Organs

Rauf, Sakandar (1), A. E. Hauser, Charlotte (2)

(1) Reserach Scientist, BESE division, King Abdullah University of Science and Technology (KAUST), Saudi Arabia.
(2) Professor of Bioscience, Division of Biological & Environmental Sciences & Engineering (BESE), King Abdullah University of Sciene and Technolgy (KAUST), Saudi Arabia.

Abstract:
Bioprinting of tissues and organs has emerged as a promising technology for applications in different fields, such as biomedical engineering and regenerative medicine, biotechnology and the pharmaceutical industry. However, current 3D bioprinting technologies face problems with the available printing inks being either unnatural, not body-like material (polymers) or natural, but not from human background and rather undefined material (alginate from algae, etc.) with significant batch-to-batch variations which has an impact on the sustainability of the bioprinted 3D structures. In addition, 3D bioprinters use a pre-polymer viscous solution to print in 3D followed by an additional crosslinking step accomplished by UV-treatment or chemical polymerization initiation in order to produce the polymerized scaffold. It also rises concerns that the cells to be printed might get damaged due to the exposure to dangerous UV light or to toxic chemicals which could compromise the cells’ viability. Our 3D bioprinting system was designed to be compatible with peptide bioinks newly developed at KAUST’s Laboratory for Nanomedicine. The components of the 3D bioprinting system include a programmable robotic arm, a custom designed extruder for bioprinting, and multiple microfluidic pumps. The robotic arm was customized as a 3D bioprinter by substituting the extruder with a designed coaxial nozzle made of three inlets and one outlet. The programmable microfluidic pumps transport the peptide ink, phosphate-buffered saline (PBS) and human cells in cell culture media through the coaxial nozzle to extrude the end product as a peptide nanogel thread. We are confident that our 3D bioprinting system will contribute to the growing advancements in 3D bioprinting and will allow the production of human-like tissues and organs for procedures such as skin replacement and tissue transplants.

Reference:

Attachment:
Figure 1: http://membs.org/membs/uploads/congress_speaker_files/1529047594abstract1_Fig.jpg
Abstract:

Background: Contemporary targeted anticancer therapy, tyrosine kinase inhibitors (TKi), have significantly improved the clinical outcomes against various malignancies. However, TKi are associated with severe cardiotoxicity. Multiple signaling pathways have been suggested to be involved in cardiotoxicity-induced by TKi. However, the role of p90 Ribosomal S6 Kinase (RSK), an important protein kinase in cell proliferation, is not fully defined. In this study, we compared the cardiotoxicity of different generations of TKi to understand the molecular mechanisms, particularly p90 RSK, associated with TKi cardiotoxicity. This will allow to identify preventative strategies. Methods: In vitro rat cardiomyoblasts (H9c2) were treated with four FDA-approved TKi, imatinib, sunitinib, dasatinib and ponatinib, to determine the cell viability (MTT), cell area using crystal violet dye, apoptosis, and p90RSK protein expression. Cell viability, cell area and flow cytometer assays were investigated following 24-hour treatment with various concentrations ranging from 2.5µM to 10µM. A time-dependent (1, 2, 4 and 6 hours) flow cytometer and immunoblotting analyses were conducted for ponatinib and sunitinib, which showed greater cardiotoxicity. Results: Sunitinib significantly reduce H9c2 cell viability by approximately 4 % and 22 %, at 2.5 and 5µM treatment concentrations, respectively; (95.68 ± 0.46 % and 78.12 ± 1.27 % of control; p < 0.01). Dasatinib demonstrated a 23 % reduction in cell viability at 5µM (77.22 ± 2.37 % of control; p < 0.01). Similarly, ponatinib at 2.5µM reduced H9c2 cell viability by 22 % (78.25 ± 3.8 % of control; p < 0.05), while ponatinib at 5µM significantly inhibited the cell viability by approximately 40 % (61.46 ± 1.49 % of control; p < 0.01). Cell area analysis demonstrated that sunitinib at 5µM following 24 hours resulted in significant increase in cell area (115.87 ± 5.27 % of control; p < 0.05). In contrast, treatment with ponatinib 2.5µM caused a significant decrease in cell area (69.23 ± 9.87 % of control; p < 0.05). No changes in cell area were seen following treatment with dasatinib and imatinib. Apoptosis was evident following 24-hour treatment with sunitinib and ponatinib at 2.5µM or 5µM. Immunoblot analysis of H9c2 cell lysates showed an increase of the ratio between cleaved caspase 3 : caspase 3 following 6-hour treatment with sunitinib 2.5µM and ponatinib 5µM. Similarly, increase of p90RSK phosphorylation was shown with ponatinib 5µM, while no change was shown following 6-hour treatment with sunitinib. Conclusion: Targeted TKi, sunitinib and ponatinib induce cytotoxic effects, which may be through inducing caspase-3 dependent apoptosis and p90 RSK.

Reference:

Engineering Specificity and Function of Therapeutic T Cells to Enhance Cancer Immunotherapy

AlSaieedi, Ahdab (1), Stauss, Hans (2)

(1) Assistant Professor, Applied Medical Sciences, King Abdulaziz University, Saudi Arabia.
(2) Professor and Director, Immunity & Transplantation, UCL, United Kingdom.

Abstract:
Adoptive cell therapy using TCR-engineered T cells is an exciting area of research and has emerged as a promising strategy for treating cancer patients. However, the effector function of TCR-engineered T cells can be tuned down by local mechanisms of tumour associated immunosuppression. The potential of cytokines to reverse local immune suppression and enhance tumour immunity has been described in the past. The main aim of this project was to engineer T cell specificity as well as effector cytokine production as a strategy to enhance cancer immunotherapy. This was achieved by combining TCR gene transfer with genetic engineering to achieve IL-12 and IL-27 production in therapeutic T cells. In vitro validation data demonstrated not only an enhanced production of IL-12 and IL-27 by the engineered T cells but also an enhanced effector function upon antigen specific stimulation. In order to circumvent previously described toxic side effects observed with systemic IL-12 delivery, a tet-regulated gene expression system was utilised to regulate cytokine production by engineered T cells in vivo. Adoptive transfer of TCR-redirected T cells expressing regulated IL-12 in B16F10 melanoma-bearing mice resulted in an enhanced accumulation of transferred CD8+ T cells in the tumour and in a change of the innate immune cell composition in the tumour microenvironment. Importantly, regulated IL-12 delivery resulted in enhanced therapeutic efficacy of the transferred T cells without causing systemic toxicity. IL-27 delivery in engineered T cells also showed some effectiveness when combined with TCR gene therapy, although the therapeutic benefit of IL-27 was inferior to IL-12. The data in this study demonstrate the potency of additional genetic manipulation to tailor the TCR-redirected T cell effector function which can result in a substantial enhancement in their therapeutic efficacy, and thus, enhanced antitumor immune response.
The E3 ubiquitin ligase, HECTD1, is involved in ABCA1-mediated cholesterol export from macrophages.

Aleidi, Shereen (1)

(1) Assistant Professor, Clinical Biochemistry, The university of Jordan, Jordan.

Abstract:
The ABC lipid transporters, ABCA1 and ABCG1, are essential for maintaining lipid homeostasis in cells such as macrophages by exporting excess cholesterol to extracellular acceptors. These transporters are highly regulated at the post-translational level, including protein ubiquitination. Our aim was to investigate the role of the E3 ubiquitin ligase HECTD1, recently identified as associated with ABCG1, on ABCG1 and ABCA1 protein levels and cholesterol export function. Here, we show that HECTD1 protein is widely expressed in a range of human and murine primary cells and cell lines, including macrophages, neuronal cells and insulin secreting ß-cells. siRNA knockdown of HECTD1 unexpectedly decreased overexpressed ABCG1 protein levels and cell growth, but increased native ABCA1 protein in CHO-K1 cells. Knockdown of HECTD1 in unloaded THP-1 macrophages did not affect ABCG1 but significantly increased ABCA1 protein levels, in wild-type as well as THP-1 cells that do not express ABCG1. Cholesterol export from macrophages to ApoA-I over time was increased after knockdown of HECTD1, however these effects were not sustained in cholesterol-loaded cells. In conclusion, we have identified a new candidate, the E3 ubiquitin ligase HECTD1, that may be involved in the regulation of ABCA1-mediated cholesterol export from unloaded macrophages to apoA-I. The exact mechanism by which this ligase affects this pathway remains to be elucidated.

Attachment:
research paper:
Investigation of Putative Substrate(s) of a Protein Kinase from Amsacta moorei entomopoxvirus

Muratoglu, Hacer (1), Demirbag, Zihni (2), Nalcacioglu, Remziye (3)

(1) Researcher, Karadeniz Technical University, Turkey, Karadeniz Technical University, Turkey.
(2) Head of Research Group, Department of Biological Sciences, Karadeniz Technical University, Turkey.
(3) Dr., microbiology, Karadeniz Technical University, Turkey.

Abstract:
Background: Amsacta moorei Entomopoxvirus (AMEV) is an insect viruses that belongs to Poxviridae family and Entomopoxviridae subfamily, and has pathogenic effect on insect pests. AMEV encodes a novel protein kinase gene (ORF AMV197), which is a homologue of poxvirus B1 protein kinase. Sequence derived amino acid analysis of this ORF suggested it to be a serine / threonine protein kinase (PK) having conserved pk and serine / threonine pk domains. It is known that Ser/Thr protein kinases of Poxviruses have roles in virus replication, morphogenesis, regulation of host cell cycle, and apoptosis. Methods: In this study, we investigated the activity of AMEV protein kinase enzyme expressed in baculovirus expression system (Invitrogen), by peptide microarray and pull-down assays. Results: We report here the functional characterization of a serin/threonine (Ser/Thr) protein kinase gene (ORF AMV197) of Amsacta moorei entomopoxvirus (AMEV). Expression of the AMV197 gene in a baculovirus expression system yielded the protein product. Pull-down assay of the AMV197 protein with the subcellular protein fractionations of Ld652 cells showed that it is using two cellular proteins (18 and 42 kDa) as novel putative substrates. Results indicated that AMV197 phosphorylates two novel putative cellular substrates belong to Ld652 cells. Our results provided that AMEV is able to regulate cellular mechanisms by phosphorylating cellular proteins through AMV197 protein kinase. However, further experiments are needed to identify the exact role of this protein kinase in the replication of AMEV.

Reference:
This study has been funded by The Scientific and Technological Research Council of Turkey (Project No: 110T887)
Abstract:

The synergistic interaction was demonstrated when human breast cancer cells MCF-7 were treated with combination of two chemotherapeutic drugs: doxorubicin (DOX) and 5 fluorouracil (5FU). 5FU and DOX were inhibit the proliferation of MCF-7 cells in a dose-dependent effect according to dose response curves that constructed for each drug alone, and the combinations in vitro. The DOX at (0.1) nM was antagonistic according to CI value that was 2.82283, while at (1) nM, the CI was 0.39429 that indicate strong synergistic action, that suggest may this combination regimen has clinical application in the breast cancer treatment.

Reference:

A novel mitochondrial DNA deletion in patient with Pearson syndrome

Rame Khasawneh *MD, FABMGG,†, Hala Alsokhni* MD,‡, Bayan Alzghoul* MD,§, Asim Momani* MD, Nazih Abualsheikh* MD, Nabeeha Abbasi* MD, Nazmi Kamal* MD, FCAP

khasawneh, rame (1)

(1) Consultant, hematology and genetics, rms, Jordan.

Abstract:

Pearson syndrome is a very rare multisystemic mitochondrial disease characterized by sideroblastic anemia and exocrine pancreatic insufficiency. It is usually fatal in infancy. We reported a four-month-old infant presented with fever and pancytopenia. Bone marrow examination showed hypoplastic changes and sideroblastic features. Molecular Study showed a novel heteroplasmic mitochondrial deletions (m. 10760 -m. 15889+) in multiple genes ( ND4,ND5,ND6, CYTB).

Reference:

entification and Association polymorphisms of TGF-b and IFN-y Genes with Schizophrenia risk in Iraqi Patients

Abed nasser, Anwar (1)


Abstract:
Abstract This study included sixty five blood samples collected from persons with mean age 20-70 years. Thirty eight blood samples were collected from persons with schizophrenia with mean age 1.6±49.95 years, and twenty seven blood samples collected from healthy as a control sample with mean age 2.4±39.7 years. Immunity and genetics study for IFN-? (Interleukin-?), T/A +874 and TGF-?1 (Transforming growth factor-beta), (+869*C/T) genes polymorphisms of associated between patients and control. Polymorphisms of IFN-? T/A +874 and TGF-?1 (+869*C/T) genes was studied by using ARMS-PCR (Amplification refractory mutation system technique). The results of IFN-? T/A +874 and TGF-?1(+869*C/T ) genes revealed the presence of two alleles, A and T and three genotypes TT, TA and AA for IFN-? T/A +874 gene, also two alleles C and T and three genotypes CC,TT and CT for TGF-?1(+869*C/T ). The result show that allele A genetics frequency more than T allele in schizophrenia patients samples of IFN-? T/A +874, and associated with etiological fraction of schizophrenia risk, dependent on values of odds ratio(OR) and confidence intervals (CI), While T allele associated with preventive fraction (PF) of schizophrenia risk. The ARMS-PCR analysis results present AA and TT homozygotes genotypes of IFN-? T/A +874 gene more than in schizophrenia patient's compression with control, and present AA and TT as genotypes associated with etiological fraction in schizophrenia risk, while TA heterozygote genotype present more than in control compression with schizophrenia patients, and associated with preventive fraction of schizophrenia risk. The results of statistical analysis of ARMS-PCR technique for the TGF-?1(+869*C/T ) gene, T allele present high frequency than C allele in schizophrenia patients, and associated with etiological fraction in schizophrenia risk, while present C allele associated with preventive fraction in schizophrenia risk. The results present TT homozygote genotype frequency of TGF-?1 (+869*C/T) gene more than in schizophrenia patients compression with control, and associated with etiological fraction of schizophrenia risk, while CC homozygote and CT heterozygotes genotypes present more than in control compression with schizophrenia patients, and associated white preventive fraction of schizophrenia risk. This results show genetics association of IFN-? T/A +874 and TGF-?1 (+869*C/T) with schizophrenia risk in Iraqi patients.

Reference:
Anwar Abed Nasser Dhabaan The Iraqi University / Collage of Education, Iraq

Attachment:

Evaluation of the performance of a new urine assay to detect prostate cancer

Bajaba, Rasha (1)

(1) Unemployed, Biotechnology, Unemployed, Saudi Arabia.

Abstract:
Prostate cancer is a considerable cause of morbidity and mortality in men worldwide. The available diagnostic methods are leading to an overtreatment and over-diagnosis for indolent tumour. Predictions of an increase in prostate cancer incidences warrant developing effective diagnostic and screening tests. The advancement in technology has paved the way for epigenetics to introduce hyper-methylation of DNA to detect aggressive prostate cancer in its earlier stages. The occurrences of methylation vary among patients, which make using one gene for detection, is quite poor. epiCaPture, the novel urine assay, detects the methylation in 6 different genes from the cell pellet fraction of urine. An evaluation of the performance of this new urine assay to stratify patients diagnosed with prostate cancer has been performed on other urine fractions from the epiCaPture study cohort; supernatant and whole urine and compared the results to cell pellet epiCaPture results. Preliminary results have shown no statistical significance (P < 0.05) between all DNA yield in all three fractions. In addition, there was no correlation between DNA concentration and DNA methylation results. However, further analysis of methylation shows highly elevated epiCaPture result in the supernatant fraction. In addition, potential result obtained from a whole urine fraction shows presence of methylation in one of the genes that was undetermined in the other fractions. Indeed, cell pellet fraction has more variety of methylated genes within the fraction than the supernatant and the whole urine. This might suggest the sensitivity of using cell pellet fraction for prostate cancer diagnosis. However, the results suggest that supernatant fraction might be useful in other prostate cancer epigenetic related research. Future research will be conducted on a larger cohort population to confirm these findings.

Reference:
The relationship between chronic supportive otitis media and enterobacteriaceae in wasit province, Iraq

Alsaidi, Muntadher (1), Hameed, Hussam (2), Faisel, Semaa (3)

(1) Associate dean for administrative affairs, Microbiology, College of medicine, Iraq.
(2) Majeed, Surgery ENT, College of medicine wasit university, Iraq.
(3) Msc student, Microbiology, College of medicine wasit university, Iraq.

Abstract:
Chronic supportive otitis media is a group of inflammatory disease of the middle ear and it is a worldwide disease, it is caused by bacterial, fungal and viral infection, the study aim to determine the relationship between Escherichia coli as enterobacteriaceae group and CSOM patients, one hundred patients were included in this study with case history of CSOM with data such as age, sex, education stage and geographical distribution, among those 100 random CSOM patients 10 of them appeared infected with E.coli bacteria, 6 male had positive result of E.coli infection and the other 4 positive result patients was female, according to the age the positive E.coli infected male was ranging between (19 – 49 years old) while the age of positive E.coli infected female was ranging between (30 – 50 years old), most of positive infected patients finished primary school and half of them are living in city others in suburb, this study shows that all patients are prudent and not involve children or teenagers.

Reference:
Abstract:

Metabolomics is "a high-throughput method" to evaluate the pathophysiological phenomenon and to classify novel biomarkers to explore molecular mechanisms of the pathological profile and evaluate drug candidate to improve drug safety and efficacy. To conduct this aim the liposome was prepared from lecithin and cholesterol 1:1 by bangham ordinary method these lipid film hydrated by aqueous phosphate buffer containing aspirin. The liposome entrapment efficiency was 85.5% multilamellar and multi-vehicles shape, with size range (165±5.82 nm). The bioassay challenge the liposome formula of aspirin and biomarker metabolomics was performed by three hundred virgin female mice. Mice grouping were two equal main groups; control and treated groups with aspirin and liposomal aspirin-treated dosing groups 1.4 and 0.7mg/kg BW for each treated group. Metabolomics was determined by a collection of ovarian tissue and extraction of the metabolites was conducted by using extraction solvent methanol:chloroform:water (1:1:2). The samples were derivatized by (MSTFA) then analyzed by GC/MS. Results showed that aspirin with 1.4 mg/kg BW had reduced estrus cycle intervals and increased the period of diestrus phase after 25 days of treatment, due to the primary persistence of corpus luteum, whereas aspirin 0.7 mg /kg BW and liposomal aspirin in two doses 0.7 and 1.4 mg /kg BW. was record fewer changes in ovarian tissue and estrus cycle phase's period. Hormonal profile indicated decreased estrogen and increased progesterone level at aspirin 1.4mg/kg BW. the other groups no significant effect on the hormone in other treated groups. Detection of 32 metabolites in ovary these were distributed in amino acids (14%), carbohydrate (43%), fatty acid (36%) and aspirin metabolites (7%).The quantity of metabolite measured according to the density of accuracy in relative level to control.Aspirin and liposomal aspirin 1.4 mg/kg caused decreased in propanoic, palmitic, glycine,alanine,eicosanoic acid with no effect on glucose level in ovarian tissue, While 0.7mg/kg BW aspirin, liposomal aspirin, and empty liposome also caused decreased in these compound but in fold change less than aspirin1.4 mg/kg BW treated group. The conclusion, liposomal aspirin have the same therapeutic effect of aspirin with a minimized side effect on ovarian tissue and uterus with own liposomal nano advantges. 2-oxopropanoate were abiomarker of aspirin so these would give an indicator of reproductive – ovarian fingerprint.

Reference:


Abstract:

Cytochrome P450 oxidoreductase (POR) is a membrane protein that exists in the endoplasmic reticulum of the cell. It is vital for electron transfer to cytochrome P450 proteins (CYPs), which use these electrons to synthesize steroids and detoxify xenobiotics. In order for POR to transfer electrons to CYPs, it has to undergo conformational changes, which requires domain motion. The details of this motion can be amplified and characterized by the so called nuclear magnetic resonance (NMR) spectroscopy. This technique requires POR to contain isotopes with spin of 1/2 such as 1H, 13C or 31P. To introduce such spins into the protein, like 13C, the protein is reacted with 13C-methyl-methanethiosulfonate (MMTS). Once, 13C-MMTS is introduced into POR, a reaction occurs between the cysteine amino acids in the protein and this reagent to generate 13C-methyl-thiocystein (MTC), which gives intense NMR signals. This project uses the MMTS reaction with POR to generate MTC signals which can be viewed with NMR to better understand POR dynamics and motion.

Reference:

Ibn El-Bitar Project

benabdellkader, sakina (1)

(1) Biologist and project leader, independent, independent, Algeria.

Abstract:
According to the results of scientific research, the premature cellular aging triggered by the oxidative stress gives rise to many diseases like: obesity, diabetes, and other health complications. This oxidative stress caused by free radicals, chemical species, extremely unstable containing an electron not paired. This compound may react by attacking the more stable molecules cells of our body to match its electron, thus speeding up the cellular aging. GM foods, meat from animals fed artificially, artificial food additives (preservatives, flavors, dyes, ...), excessive consumption of drugs and emotional stress is the main source of free radicals. To address this serious problem of free radicals, we tried to have a solution by production of the nutraceutical products based on medicinal plants, rich in natural antioxidants that they have the ability to inhibit the free radicals by creating the covalent addition, thus inhibits the triggering oxidation chain reactions and stop their propagation.
Expressions of CD markers immunophenotyping by flow cytometry among pediatric population reported at children hospital and institute of child health

khan, faiza (1), khan, faiza (2)

(1) Medical Lab Technologist, Department of Medical Sciences, Chughtais Lahore Lab, Pakistan.
(2) Medical Lab Technologist, Department of Medical Sciences, Chughtais Lahore Lab, Pakistan.

Abstract:
Please find attached file

Attachment:
Abstract file:
http://membs.org/membs/uploads/congress_speaker_files/1525010380ABSTRACT sahs.docx

Abstract presentation:
http://membs.org/membs/uploads/congress_speaker_files/1525010380Faiza Khan.pptx

Cv:
http://membs.org/membs/uploads/congress_speaker_files/1525010380cv to send.docx
Meta- Molecular Medicine (MMM) and the Coming Medical Revolution

Rizk, Mustafa (1)

(1) Consultant physician & Energy medicine activist, Private practice, Private, Egypt.

Abstract:
Extending scientific researches beyond the molecular level into the subatomic one means the dawn of a new revolution in medicine for answering the eternal question who we are and how our bodies work? Researches in the field of Nano biology have confirmed that a living organism works through an informationatechnology. All bio-components: cells, molecules, atoms, and electrons protons and neutrons have some sort of mind to work together to run the organism as a whole. Wet proteins are semiconductors with channels, gates and switches for the circuiting free electrons. The connective tissue with its predominant protein: collagen connects skin to intercellular, intracellular and nuclear compartments of every nook and cranny in the body constituting an extensive network labeled as the living matrix which has other biophysical properties such as piezoelectricity. Biological water has been discovered to have memory retaining the EM signature of the previously immersed molecules. The concept of informational biofield has been added depending on the measurable electromagnetic activity of living tissue. Consciousness has regained its superiority again and many researches are underway in the area of mind to body and mind to mind interrelationship.

Reference:
References 1 -The Bofield Beverly Rubike 2 -Spontaneous Healing Andrew Well 3 -The One mind larry dossey 4 -DNA waves and Water Luc Montagnier 5 -Nanoplasm the Utimate Unit of Lfe Gilbert Ling 6 -Tensegrity and Mechanotransduction Donal Ingeber 7 -Energy Medicine and Therapeutic Performance James Oschman 8 -Rainbow and the Worm Mae Wan Ho 9 -Biology of Belief Bruce Lepton
H2AX-tyrosine 142 phosphorylation plays a role in determining cell fate in conjunction with phosphorylated H2AX-serine 139 in the dormancy enriched leukaemic cell line (KG1a) post DNA damage

ALDosari, Sahar (1), ALDosari, Sahar (2), ALDosari, Sahar (3), ALDosari, Sahar (4)

(1) Senior Laboratory Technologist, Cytogenetic and Molecular Genetics, Prince Sultan Military Medical City, Saudi Arabia.
(2) Senior Laboratory Technologist, Cytogenetic and Molecular Genetics, Prince Sultan Military Medical City, Saudi Arabia.
(3) Senior Laboratory Technologist, Cytogenetic and Molecular Genetics, Prince Sultan Military Medical City, Saudi Arabia.
(4) Senior Laboratory Technologist, Cytogenetic and Molecular Genetics, Prince Sultan Military Medical City, Saudi Arabia.

Abstract:

Background: The clonal disorder acute myeloid leukaemia (AML) is a devastating and mostly incurable disease characterized by the accumulation of poorly differentiated myeloid blast cells in the bone marrow and peripheral blood. Dormant leukaemic initiating cells (DLICs) are a small population of cells with stem cell characteristics that reside at the top of the leukemic hierarchy, like HSCs in hematopoiesis. Methods: CD34+CD38- KG1a AML cell line was grown with rapamycin to produce dormancy-enriched cell populations. The DNA damage scored by neutral comet assay. The DNA damage response (DDR) was measured by flow cytometric analysis (FACS) of phosphorylated ATM-S1981, H2AX-S139 (?H2AX), H2AX-Y142 and Chk2-T68 pre and post damage induction by 20µM etoposide and 1µg/ml ara-c for 24 hours. Results: Analysis of ?H2AX-S139 shows significant difference in ?H2AX phosphorylation between dormancy enriched cells and cycling cells after ara-c 1µg/ml (p=0.018)* and 20µM etoposide (p=0.027)* for 24 hours (n=12). Reduced in H2AX-Y142 phosphorylation influences the recruitment of ?-H2AX that indicate activation of DNA repair pathway in dormancy enriched cells and exert resistance to apoptosis compared to cycling cells. Conclusion: These results may enable to understand the efficiency and/or limitations of conventional chemotherapy and to identify a novel treatment strategies to eradicate both dormant and actively proliferating leukaemic cells permanently.
Multiple drug resistance and biocide resistance in Escherichia coli environmental isolates from hospital and household settings.

Ghanem, Bothyna (1), Haddadin , Randa (2)

(1) Lecturer, College of Pharmacy - Pharmaceutical Sciences Section, Amman Arab University, Jordan.
(2) Associate professor of Pharmaceutical microbiology, Pharmaceutics and pharmaceutical technology, The University of Jordan- School of Pharmacy, Jordan.

Abstract:

BACKGROUND: Antibiotic resistance of environmental Escherichia coli in hospitals could be increased due to extensive use of biocides resulting in serious infections. In this study, the prevalence of antibiotic resistance of environmental isolates of E. coli from hospitals and household settings were evaluated and compared. In addition, the association between biocide minimum inhibitory concentration (MIC) and multiple drug resistance (MDR) was investigated. METHODS: Environmental samples were collected from different homes and hospitals in Amman, Jordan. The isolates were identified phenotypically and by PCR. Antibiotic susceptibility tests and MIC of selected biocides were performed on the isolates. Screening for blaCTX-M group 1 was also performed. RESULTS: Of 21 E. coli strains isolated, 47.6% were MDR and 67.9% were phenotypically identified as extended spectrum beta-lactamase (ESBL) producers. The occurrence of these ESBL isolates was comparable between household and hospital settings (P>0.05). The MIC values of the biocides tested against all isolates were well below the in-use concentration of biocides. Moreover, the MICs of biocides were comparable between isolates from households and those from hospitals (P>0.05). No association was found between MDR and biocide MIC (P>0.05). Most of ESBL isolates harboured blaCTX-M 1. CONCLUSIONS: The extensive use of biocides in hospitals is not associated with MDR nor does it affect the MIC of biocides against E.coli.

Attachment:

**Evaluation of Transforming Growth Factor-beta1 and Haptoglobin mRNAs in Blood of Prostate Cancer Patients**

sherif, mohammed (1)

(1) Medical investigation, Biochemistry, National Cancer Institute, Egypt.

**Abstract:**

- Compare the levels and the diagnostic efficacy of the mRNA of TGF-β1 and Hp in the whole blood between newly diagnosed patients with prostate cancer, patients with benign prostatic hyperplasia (BPH) and Normal individuals and their relation with some of the clinicopathological findings of prostate cancer.  
- Evaluate the correlation between TGF-β1 and Hp in the studied groups.  
- Compare the utility of TGF-β1 and Hp with that of PSA that is routinely used for the diagnosis and follow up of patients with prostate cancer in order to find more sensitive markers for prostate cancer

**Reference:**

Meta- Molecular Medicine (MMM) and the Coming Medical Revolution

Rizk, Mustafa (1)

(1) Consultant physician & Energy medicine activist, Private practice, Private, Egypt.

Abstract:

Extending scientific researches beyond the molecular level into the subatomic one means the dawn of a new revolution in medicine for answering the eternal question who we are and how our bodies work? Researches in the field of Nano biology have confirmed that a living organism works through an informationatechnology. All bio-components: cells, molecules, atoms, and electrons protons and neutrons have some sort of mind to work together to run the organism as a whole. Wet proteins are semiconductors with channels, gates and switches for the circuiting free electrons. The connective tissue with its predominant protein: collagen connects skin to intercellular, intracellular and nuclear compartments of every nook and cranny in the body constituting an extensive network labeled as the living matrix which has other biophysical properties such as piezoelectricity. Biological water has been discovered to have memory retaining the EM signature of the previously immersed molecules. The concept of informational biofield has been added depending on the measurable electromagnetic activity of living tissue. Consciousness has regained its superiority again and many researches are underway in the area of mind to body and mind to mind interrelationship.

Reference:

1 -The Bofield Beverly Rubike 2 -Spontaneous Healing Andrew Well 3 -The One mind larry dossey 4 -DNA waves and Water Luc Montagnier 5 -Nanoplasm the Utimate Unit of Life Gilbert Ling 6 -Tensegrity and Mechanotransduction Donal Ingeber 7 -Energy Medicine and Therapeutic Performance James Oschman 8 -Rainbow and the Worm Mae Wan Ho 9 -Biology of Belief Bruce Lepton
In Vitro Agricultural Products, Food Security and Bio-safety for Health Security

Ahmed Abdul, Bakrudeen Ali (1)
(1) Associate Professor, Dept of Biotechnology and Biochemistry, PRIST UNIVERSITY, India.

Abstract:
Agriculture is expected to feed an increasing population, forecasted to reach 8 billion by 2020, out of whom 6.7 billion will be in developing countries where the carrying capacity of agricultural lands will soon be reached. Genome-editing technology has been widely used in medicine, animals and agriculture. Genome-editing techniques with sequence-specific nucleases (SSNs) creates DNA double-strand-breaks (DSBs) in the genomic target sites that lead to gene mutations, insertions, replacements or chromosome rearrangements by non-homologous end joining (NHEJ) or homology-directed repair (HR) mechanisms. Plant biotechnology comprised only a few applications of tissue culture, recombinant DNA technology and monoclonal antibodies. Today, genetic transformation, and marker-aided selection and breeding are just a few of the examples of the applications in crop improvement with profound implications around the world. Plant biotechnology applications must respond to increasing demands in terms of food security, socio-economic development and promote the conservation, diversification and sustainable use of plant genetic resources as basic inputs for the future agriculture of the Region. Food security is defined by FAO as the access by all people at all times to the food needed for a healthy and active life. The concept means the achievement of the food self-sufficiency, and guarantees that this condition will be sustained in the future. Food security implies reaching productive growth and the preservation of the environment. Plant tissue culture offers a viable alternative for propagation via somatic embryogenesis and plant regeneration. Plant biotechnology offers several possibilities for increasing productivity, diversification and production, while developing a more sustainable agriculture. It includes bio-pesticide production, plant tissue culture techniques, and the use of advanced molecular biology techniques for plant transformation, genomic analysis coupled with breeding and plant-disease diagnoses. Many small research teams in universities or agricultural institutions, poorly connected and/or integrated, have a high dispersion of facilities and qualified labor force. The demand for ornamental and medicinal plants is therefore very high leading to their over-exploitation from the wild population. Therefore, an efficient propagation protocol needs to be developed for this species for its application in traditional medicine and commercial planting. Beside that the comparative approach focusing on the determination of similarities and differences between the genetically modified food and its conventional counterpart aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods. The USDA is the leading US regulator under the coordinated framework and provides guidance on how to regulate modern biotechnology products to technology developers followed by the Animal and Plant Health Inspection Service (APHIS) at US. FDA’s consideration is focused on product-base, and holds the position of considering foods and feeds derived from genetic engineering technology as safe as their conventional counterparts following voluntary consultation process. Regulation related to genetic engineering technology within EPA is mainly focused to insect resistant traits. As well, the Canadian Regulatory Framework for Biotechnology, the basis of Canada's regulation of biotechnology, is triggered primarily by the “product” and its novel trait. The definition of a novel trait is given by the Canadian Food Inspection Agency (CFIA), where plant with a novel trait (PNT) developed from conventional breeding, mutagenesis, transgenesis or genome editing will all be subject to a similar regulatory approval process and are regulated by the Canadian Food Inspection Agency in cooperation with Health Canada. The sale of foods derived from these PNTs is controlled by Health Canada via the mandatory pre-market notification requirement. An herbicide-tolerant variety of canola developed by Cibus Global was approved for use in Canada by the Canadian Food Inspection Agency and Health Canada in March 2014. It is the first commercial crop generated using genome editing. In Argentina, National Commission Advisor in Agricultural Biotechnology (CONABIA) shall perform the assessment for each NBT-derived crop submitted by applicants to see if the result of the breeding process is a new combination of genetic material. The European Academies Science Advisory Council (EASAC) concluded in 2013 that “the trait and product, not the technology, in agriculture should be regulated, and the regulatory framework should be evidence-based”, which was also endorsed by the academies. EASAC also asked EU regulators to confirm that the products of new breeding techniques. Finally, as a very promising technology, genome editing not only has powerful applications in crop improvement and animal health but has also been widely used in drug discovery, which is more closely related to human health and life Keywords: In vitro techniques, Agricultural Products, Food Security, GMO Plant, Bio-safety assessment, Health security.

Reference:
Attachment:
Association of hsa-miR-145-5p and SCL1A2 gene in Brain Tissue

Samani, Samaneh (1), Ghaedi, Kamran (2)

(1) master of Genetic, Nourdanesh institute of higher education, Division of cellular and Molecular biology, Department of biology, Nourdanesh institute of higher education, Meymeh, Iran, Iran.

(2) Associate Professor. Mol. Cell Genetics, Cell & Molecular Biology Dept, Royan Institute and University of Isfahan/Division of Cellular and Molecular Biology, Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran, Iran.

Abstract:

Background: MicroRNAs are noncoding RNAs, which they have 18-25 nucleotides, also in most of vital biological pathways they act as oncogenes or tumor suppressors [1]. In this study, we have investigated about hsa-miR-145-5p and according to studies have been done, it has an important role in cell proliferation and angiogenesis [2]. With Bioinformatics tools it is offered the Brain is the most related tissues of hsa-miR-145-5p, also the SCL1A2 has the highest expression in this organ.

Material and Methods: The miR2Disease (www.miR2Disease/searchDiseasePre.jsp), miRBase (www.mirbase.org) and miRwalk2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/mirRetsys-self.html) databases were used respectively to predict the putative target genes with hsa-miR-145-5p. In addition the most related tissues and pathways were obtained from Peppsy (http://peppsy.genouest.org/result) and David (https://david.ncifcrf.gov/summary.jsp) databases.

Results: It is predicted the most related organs is Brain (Fig. 1) And due to our studies the SCL1A2 has a significant expression in Brain. Fig. 2 Furthermore, hsa-miR-145-5p with interrupt in Axon Guidance Pathways can be act as a disruptive factor Axon outgrowth Fig. 3. Conclusion: The microRNAs are related to many organs, according to our outcomes, hsa_miR_145_5p is connected to Brain more than other tissues. hsa-miR-145-5p by removing some factors makes a disturbance in Axon outgrowth. Many genes have expressed in the Brain, but the SCL1A2 gene in addition to the highest expression is involved in many disorders like Huntington (HD) [3].

Reference:


Attachment:

Fig.1 The most Related tissues of hsa-miR-145-5p by Bioinformatic Results : http://membs.org/membs/uploads/congress_speaker_files/1525540673pic1 (2).png

Fig.2 Total EST in pool (the most related Genes To Brain): http://membs.org/membs/uploads/congress_speaker_files/1525540673pic1 (1).png

Fig.3 Axon Guidance signaling pathways with hsa-miR-145-5p targetome: http://membs.org/membs/uploads/congress_speaker_files/1525540673s.png
Abstract:

Human Brucellosis is a zoonotic disease transmissible to human by infected animals, caused by Gram-negative Cocobacilli facultative intracellular pathogen called Brucella. There are six types of Brucellae are compromising human health two of them are endemic in Saudi Arabia according to the life style of Saudi people Brucella abortus and Brucella melitensis, since the Brucella clinical picture is not clear according to the non-specific symptoms of the infection and the difficulties on the laboratory diagnosis by the available techniques, immunological examination of the Brucellosis like serum agglutination test and ELISA shows cross reactivity with other Gram negative bacteria on other hands the Gold standard test of Brucella identification is bacterial culture which time consuming and shows false negative result in the relapsed and previously treated by antibiotic cases, also culture of Brucella could threats laboratory stuff by its ability to transmittable to human by aerosol. Molecular diagnosis is rapid technique show more accuracy higher sensitivity with time-saving approximately 6h and better in the safety of the laboratory stuff more than other available techniques. This proposal aims to enhance of Brucella diagnosis with RT-PCR that appear as the more accurate technique that able to identify the infection by each species-specific Brucella type alone, though more accuracy small quantity of the sample up to 10 fg, a shorter time than culture technique maximum 6h without any danger of contamination by Brucella spp. Subjects and Methods Study subjects: Acute Febrile Illness (AFI) cases will be recruited from Fever Hospitals. Suspected cases of brucellosis will be collected from jeddah city and allocated according to the inclusion criteria which are age, sex and family history of brucellosis or contact with animals known to have Brucella infection. Isolation of DNA from clinical blood samples: Blood samples will be collected in EDTA, stored at -20°C, and DNA will be extracted according to the standard protocols described previously. DNA amplification: A 223-bp fragment from the conserved region of the gene which encodes an immunogenic membrane protein of 31 kDa of B. abortus specific to the Brucella genus and present in all its biovars will be amplified using the B4 and B5 primers probe. RT-PCR products will be resolved automatically, and Brucella DNA detected according to their molecular size. This test will be used to confirm the Brucella positive samples which will be subjected to the species-specific DNA amplification. Species-specific DNA amplification: Species-specific DNA segments of B. abortus and B. melitensis will be amplified using specific primers derived from the IS711 element (Gen Bank accession#M94960). The forward primer spans 803 to 823 nt of IS711 and generates a 113-bp PCR product with B. abortus reverse primer, and 252-bp PCR product with B. melitensis reverse primer. The outcome of this study: The outcome of this study will appear with faster, safer, more accurate and higher sensitivity diagnosis with ability to detect Brucella infection in the early stage of the disease and in the relapses cases thus shows false negative when use culture and serology tools with low percentage could be reach 0% for cross-reactivity with other Gram-Negative Bacteria.

Reference:

Dietary protein and thermal acclimation induces biochemical stress in the liver and white muscle of Nile tilapia

salaah, sally (1)

(1) Post Doctoral Researcher, Physiology Department, NIOF, Egypt.

Abstract:
This study investigated the effects of dietary protein and water temperature on biochemical parameters related to oxidative stress in the liver and white muscles of Nile Tilapia (O. niloticus). Practical diets were formulated to contain two protein levels (17% and 25%), and each diet was randomly assigned triplicate groups of 15 fishes at three temperatures (17°C, 26°C, and 36°C). Fish feed on high protein ratio and acclimated at high temperature showed significant increase in liver and white muscle antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR)]. Non-enzymatic antioxidants [Non-protein thiol groups (NPSH)], and oxidative stress biomarkers [PCO and LPO]. Moreover, (LPO/CAT+GPx) ratio was significantly high in liver and white muscles of Nile Tilapia. Indicating that fish were in stressful conditions, lead to oxidative stress.
Effect of Aluminium Chloride Intoxication on Selected Biochemical –hormonal Parameters and histopatholical in Experimental Animals

hadjer, bekhedda (1), abbessia, demmouche (2)
(1) Laboratory Scientist/PhD student, biology, university , Algeria.
(2) profesor, Dept.biology, university djilali liabes , Algeria.

Abstract:
We are currently living in the "aluminum age" and its exposure is inevitable. Today, its toxicity is well established in animals, and an epidemiological link has indeed been demonstrated between its exposure and neurodegenerative diseases, following an overproduction of free radicals responsible for damage to the nervous system and other organs. The present study evaluates, biochemical and hormonal disturbances, Aluminum chloride administered to female rats (intraperitoneally10 mg/kg body weight) at a daily for 5 D.10D 15D. resulted in a significant reduction in body weight. The histological study showed alterations in the cerebral cortex and liver marked by cellular degeneration. Serum exploration of biochemical parameters revealed that aluminum causes disruptions in the activity of enzymes such as (gly. ALT, AST,) in the liver and (creatinine and urea) at the renal level sign of dysfunction. The impact of aluminum on the hormonal balance showed an increase in the levels of different hormone in rats intoxicated by aluminum.
Impact of chemotherapy on the alteration of the hepatic assessment of cancerous subjects in the city of Sidi Bel Abbes

hadjer, bekhedda (1), abbesia, demmouche (2)

(1) Laboratory Scientist/PhD student, biology, university, Algeria.
(2) profesor, Dept. biology, university djilali liabes, Algeria.

Abstract:

Hepatic tolerance of anticancer drugs is a topical issue related to both the intrinsic toxicity of certain cancer chemotherapies and the terrain represented by the cancer patient himself. The hepatotoxicity of these molecules may be revealed by hepatic biochemical markers. This work is a prospective, analytical study of chemotherapy patients in 196 women and 104 men, mean age 56, with a cancerous disease, of which breast cancer represents 42% of cases. These patients undergo cancer chemotherapy and are followed for four courses of treatment. Mean levels of hepatic TGO, TGP, alkaline phosphatase, Gamma-GT, and total and direct bilirubin levels and their extremes increased by norms during the course of the four courses of chemotherapy. These rates rise after each treatment significantly. Transaminases appear to be sensitive and specific markers of hepatocytolytic involvement. In addition, the mean liver enzyme values were higher after the 4th course. Some hepatic pain may have developed after several cures causing some hepatic cytotoxic or cholestatic hepatic toxicity. In conclusion, it can be said and probably that the antimitotic products used can probably cause hepatic toxicity, because the adaptation of dosages and established prevention measures are insufficient.
Environmental sampling of Staphylococcus aureus at a Large, Midwestern Liberal Art College: molecular epidemiology at the intersection of non-healthcare and healthcare facilities

Younus, Mohammed (1), Smith, Tara (2)

(1) Head of the Molecular Biology Lab, Bio-detection, ministry of science and technology, Iraq. (2) Head of the Smith Emerging Infections Laboratory, Biostatics, Environmental health science & Epidemiology, Kent State University / College of Public Health, United States.

Abstract:

Staphylococcus aureus is a commonly found bacterium that colonizes a variety of animal species, including humans. Additionally, it is also found on fomites and has the potential to be transferred from person to person via contaminated surfaces. Methicillin-resistant S. aureus strains (MRSA) first emerged in hospital settings (HA-MRSA), but are also found in the community (CA-MRSA). With over 11,000 deaths from MRSA and the ability to survive on surfaces for several months, we characterized environmental contamination of both healthcare and non-healthcare associated college student buildings in order to determine molecular type and antibiotic susceptibility profiles of S. aureus isolates. Environmental swabs (n=152) were collected from healthcare (n=2) and non-healthcare-associated buildings (n=2) at a state university. A total of 38 samples were collected per building from high hand-touch areas (door handles, sink handles, etc.) and processed within 24 hours using typical bacteriological methods. Five colonies per positive sample were then subjected to antibiotic susceptibility testing and molecular characterization (multilocus sequence typing, PVL and mecA PCR, and spa typing). A total of 34 of 152 (22.4%) samples were contaminated with S. aureus and 9 (5.9%) were positive for MRSA. Three sites were positive from multidrug resistant (MDR) strains. Within healthcare and non-healthcare facilities, 5/75 (6.75%) and 4/75 (5.26%) were MRSA positive, respectively. The most frequently contaminated surface for MRSA in buildings frequented by healthcare-associated students was the main door, whereas in non-healthcare buildings it was the classroom. All isolates were resistant to penicillin, with methicillin resistant (26.5%) and MDR (8.8%) present. The most common spa types found were t1149, t068, t216, t008, and t334. These results point to the ease of S. aureus contamination on fomite surfaces and the presence of t008 (a community-associated strain type) in healthcare-associated student buildings, along with MDR S. aureus indicates a need for increased awareness of the potential for environmental surfaces, even outside of the hospital setting, to act as reservoirs for S. aureus.

Reference:


Attachment:
Antibiotic susceptibility profile for all environmental samples.: http://membs.org/membs/uploads/congress_speaker_files/1525813173Antibiotic_resistant.docx
Prevalence of S. aureus in environmental sampling. HA= Hospital associated. NHA= Non-hospital associated.
MRSA- methicillin resistant S. aureus.


Table 1. S. aureus epidemiology across environmental sampling sites:
http://membs.org/membs/uploads/congress_speaker_files/1525813173Table 1. S. aureus epidemiology across environmental sampling sites.docx

. Environmental surface snapshot of S. aureus isolates Based-Upon Repeat pattern (BURP) analysis. The spa type with the highest founder-score is defined founder of the cluster (blue color). spa type t8337 & t548 both represent the same highest-founder sco:
http://membs.org/membs/uploads/congress_speaker_files/1525813173BURP.docx
Methods: The human DPYD gene was investigated in dbSNP/NCBI, 273238 SNPs were found; 99645 SNPs were Homo sapins; of which 534 were missense SNPs. Missense SNPs were selected for in silico analysis; SIFT, Polyphen2, SNPs & GO, Imutant 2.0, Mutation 3D, UCSF Chimera and HOPE were used to investigate the effect of SNPs on DPD protein’s structure and function.

Results: 69 SNPs were found to be highly damaging for the protein by SIFT and Polyphen, of which 4 SNPs were observed to be associated to clinical presentations (M166V, V335L, I560S, D949V). These 69 SNPs were further analyzed by SNPs & GO, one SNP (D949V) was observed to be associated to clinical presentations.

The 4 nsSNPs that observ

gharib, ali (1)

(1) teacher assistant, Department of Biotechnology, Omdurman Islamic unversity, Sudan.

Abstract:

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Reference:


Attachment:

Abstract:

Abstract Background: The aim of the present study is to investigate the chromosomal aberration in peripheral blood and solid tissue in breast cancer and using new method for detection of HER-2nue gene amplification by FISH technique to confirm the positivity of HER-2nue. Methods: 82 patients were admitted to the King Hussein cancer center with breast cancer. They detected chromosomal aberration and HER-2nue gene amplification by Immunohistochemistry and FISH technique. Results: 28 cases were positive by immunohistochemistry, 5 cases inconclusive, and 49 cases were negative. The 5 inconclusive cases by immunohistochemistry were detected by FISH technique. 3 cases were positive and 2 cases negative. The abnormality of chromosome appears in many cases in chromosome 13, 17, and 16 like Karyotype 45,xx,-16,-17 also 51,xx,+4,+8,+11,+13,+19. Conclusion: the breast cancer patients must detected for HER-2nue gene amplification by FISH technique also chromosomal aberration to chose the right therapy. Keywords: breast cancer, FISH technique, HER-2nue and chromosomal aberration.
SEVERE VITAMIN D DEFICIENCY AMONG PREGNANT WOMEN AND THEIR NEWBORNS IN SIDI BEL ABBES REGION, ALGERIA

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(2) Faculty of Natural Sciences and life-DjillaliLiabes University Sidi Bel Abbes. Algeria., Department of Biology, Biotoxicology laboratory. Department of Biology, Faculty of Natural Sciences and life-DjillaliLiabes University Sidi Bel Abbes., Algeria.
(3) Faculty of Natural Sciences and life-DjillaliLiabes University Sidi Bel Abbes., Department of Biology, Biotoxicology laboratory. Department of Biology, Faculty of Natural Sciences and life-DjillaliLiabes University Sidi Bel Abbes. Algeria., Algeria.

Abstract:

Background: Vitamin D deficiency is common in pregnant women and newborn infants. Adequate vitamin D concentrations during pregnancy are necessary to ensure appropriate maternal responses to the calcium demands of the fetus and neonatal handling of calcium. There are few data from Algeria on serum 25(OH) D concentration and the prevalence of vitamin D deficiency in pregnant women and their newborn. This study was undertaken to determine the prevalence of maternal and fetal hypovitaminosis D in SBA region of Algeria and to study their correlations with the levels of calcium, serum 25(OH)D, albumin, alkaline phosphatase (ALP) and plasma parathyroid hormone (PTH). Methods: Maternal serum and cord blood levels of calcium, 25-hydroxyvitamin D (25(OH)D), ALP, PTH were studied in 100 mother-neonate pairs at term. Results: About 86% of patients have serum 25(OH)D <3 ng/ml, and 78% of newborn have serum 25(OH)D <3 ng/ml. The mean level of maternal PTH was 92.13±45.29 pmol/L, and that of cord blood PTH was 13.61±11.82 pmol/L. The intake of calcium was low in Algerian women (796,30±366,35 mg Ca/d). The mean level of maternal serum calcium was 84.64±4.04 mg/L, and that of cord blood calcium was 96.26±9.90 mg/L. Maternal serum calcium was correlated with cord blood calcium (r=0.16, p=0.69). The mean level of maternal serum albumin was 30.84±3.26 g/L. The mean birth weight of the newborn was 3434.±437.81g (2600-4500g). Women deficient in vitamin D had infants with vitamin D deficiency respectively (4.33±4.36 ng/ml; 4.31±4.81 ng/ml). Maternal vitamin D status during pregnancy has been shown to be associated with neonatal vitamin D. Maternal serum 25(OH)D correlated positively with cord blood 25(OH)D (r=0.78, p<0.01) and negatively with PTH (r=-0.24, p<0.001). A positive linear relationship was found between gestity and maternal calcium (r= -0.32, p=0.02). Conclusion: There is a high prevalence and severe of vitamin D deficiency in pregnant women and neonates in SBA, Algeria. The newborn serum 25(OH)D concentrations rely on maternal vitamin D status. Poor maternal vitamin D status may adversely affect neonatal vitamin D status. Supplementation is needed to improve maternal and neonatal vitamin D and calcium nutrition. Keywords: Maternal vitamin D; neonatal 25-hydroxyvitamin D; parathyroid hormone; calcium.

Reference:


Attachment:

The Effect of prolonged formalin fixation on the staining characteristics of archival human brain tissue

Al-Rafiah, Aziza (1), Alshali, Rasha (2)

(1) Assistant Professor, Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, Head of Neuroscience Research Unit, Medical school, Department of Medical Laboratory Technology, King Abdulaziz University, Saudi Arabia.

(2) Assistant professor at KAU, Anatomy Department. The head of Anatomy Department, Member of Neuroscience Research Unit, Anatomy Department, King Abdul Aziz University, Saudi Arabia.

Abstract:

Back ground /objective: Neurodegenerative disorders include wide range of conditions, which affect millions of people worldwide. Unfortunately, they are incurable and irreversibly progressive. For both routine diagnostic and research purposes histological studies are done as large amount of brain tissues are stored but little is known about whether they are suitable for retrospective studies. The study aimed at investigating the effects of prolonged formalin fixation time on immunohistochemical expression of some common neurodegenerative markers in archival brain specimens. Materials and Methods: Twenty brain specimens were obtained from human cadavers in the Anatomy Department of King Abdul Aziz University, that were prefixed in 10% formalin. They were divided into two equal groups according to time of fixation, Group: 1 less than one year, Group 2: Up to 20 years. Histological examination of white and gray matter was done using H & E, Luxol fast blue (LFB) for myelin staining, Congo red for amyloid plaques, CD 68 for microglial cells, Tenascin-C (large extracellular matrix glyco protein) and Caspase 3 antibody for apoptotic cells . Results: For both groups, corpus callosum sections displayed myelination with LFB staining The distribution of CD 68 positive microglial cells was evident in frontal, temporal grey matter not in corpus callosum sections. Strongly positive masses were seen in Congo red stained frontal and temporal sections. Anti- Caspase 3 immunostaining revealed positively stained neurons. Conclusion: Histological and immunohistochemical techniques yielded reproducible staining results when applied to human brain tissue stored in formalin for long periods so they can be used in well preserved biobank material which are the most targeting research areas in neuropathology. Key words: Human Brain, immunohistochemistry, formalin fixed, neurodegenerative markers.

Reference:

Effect of Microwave Antigen Retrieval Technique on immunohistochemical staining of Human Brain Biopsies Exposed to Prolonged Formalin Fixation Time

Al-Rafiah, Aziza (1), Alshali, Rasha (2)

(1) Assistant Professor, Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, Head of Neuroscience Research Unit, Medical school, Department of Medical Laboratory Technology, King Abdulaziz University, Saudi Arabia.
(2) Assistant professor at KAU, Anatomy Department. The head of Anatomy Department, Member of Neuroscience Research Unit, Anatomy Department, King Abdul Aziz University, Saudi Arabia.

Abstract:

Background: Formalin fixed tissues, especially for longer periods, produces strong cross-links; resulting in limitation of antibodies permeation into the tissue, altering the visualization of the epitopes by immunohistochemistry (IHC) and decreasing the accessibility of antigenic determinants. To our knowledge, there are fewer studies on the effect of formalin fixation time on tissue antigenicity in human brain tissue. Moreover, microwave technology is used beneficially in many histological procedures to reduce incubation time of fixation and staining and improving the tissue morphology. Objectives: is to determine the useful effect of microwave antigen retrieval (AR) method on IHC staining of some neurodegenerative markers in formalin preserved human brain biopsies for a longer time, even up to several years. Materials & Methods: The tissue samples for this study were obtained from the frontal lobe of 16 preserved formalin brains, which were obtained from the Dissection lab of the Department of Anatomy, King Abdulaziz University. According to the lab files, the preservation time ranged from 2-3 years ago. Each brain sample was divided into two groups of small pieces (group A: procedure with microwave irradiation (5 s on/5 s off at 200W) and group B: procedure without microwave irradiation). Paraffin blocks from both groups were prepared and subjected to IHC (avidin-biotin complex interaction) to detect certain types of proteins using two specific primary antibodies (Tenascin-C and Cd68). Stained sections were examined, photographed and subjected to histometric evaluation. Results: Better IHC staining results with preserved architecture outcome were obtained with both Tenascin-C and Cd68 specific antibodies (markers for myelin) in microwave assisted brain samples even after years of formalin fixation when compared with those which were not assisted. Conclusion: Microwave assisted AR is a useful technique for immunohistochemical analyses for old brain biopsies. These results will open the door for many comparative human studies especially in the field of neurodegenerative diseases.

Reference:


van der Loos CM. A focus on fixation. Biotech Histochem 2007;82: 141-54


Title: Monocyte subpopulations in ischemic stroke patients exhibit pro-angiogenic phenotype rather than pro-inflammatory phenotype

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(1) Assistant Professor, Medical Laboratory Technology Department, Faculty of Applied Medical Sciences, Head of Neuroscience Research Unit, Medical School, Department of Medical Laboratory Technology, King Abdulaziz University, Saudi Arabia.

(2) Assistant Professor of Physiology, Department of Physiology, Faculty of Medicine, Member of Neuroscience reach unit, King Abdulaziz University, Saudi Arabia.

Abstract:

Introduction: Stroke is characterized by rapid development of focal clinical signs that indicate loss of the functioning brain, for more than 24 hours or causing the death of the patient, with unclear cause rather than of vascular source. Different monocytes subpopulations have a distinct role as phagocytic subsets but also act as pro-inflammatory and pro-angiogenic subsets. We hypothesised that monocyte subpopulations will be skewed in stroke patients. Methods: Ischemic stroke patients were recruited after diagnosis was confirmed by medical consultants. The volunteer persons, who were matching for age and sex, were participated as the control group. Fresh blood was collected after participants consent to their enrolment, and was analysed by flow cytometry for total peripheral blood mononuclear cells (PBMCs), total monocytes, and monocyte subpopulations which were identified according to the CD45, CD14 and CD16 monoclonal antibodies staining. Results: The total PBMCs and monocytes did not significantly differ amongst study groups whilst the monocytes subtests were significantly skewed. Compared to controls, the proportion of intermediate monocytes (pro-angiogenic phenotype) with the CD14highCD16+ was higher in stroke patients (P<0.001) while non-classical monocytes (pro-inflammatory phenotype) with the CD14lowCD16+ was lower (P<0.01), whereas the classical monocytes with the CD14highCD16- was unchanged. Conclusion: the results of the study are beneficial not only for a scientific researcher but also for practice of clinical neurology. It could be an early diagnostic marker to start either a neurovascular repair therapy or improve medications attributed to highly senescent subpopulations.

Reference:

Development and anticancer activity of resveratrol loaded polylactic - coglycolic acid (PLGA) implants against breast cancer implanted in mice

Al-Saadoon, Eilaf (1)

(1) Master degree researcher, Pharmaceutical science, applied science university, Jordan.

Abstract:
Breast cancer is a solid tumor and the primary cause of cancer mortality in women. One of the main problems in treating solid tumors is the low penetration of anticancer drugs in tumor tissue. Thus, an increase in the concentration of the anticancer drug may increase the efficiency of the therapy, but the toxicity associated with the use of high drug concentration is a limiting factor. Local administration of a polymeric biodegradable implant containing an anticancer drug may be an effective method of increasing drug concentration near the tumor site. The aim of our study was to develop and test resveratrol loaded polylactic - coglycolic acid (PLGA) implants as an anticancer therapy against breast cancer implanted in mice. Melt casting method was used to prepare PLGA implants loaded with various concentrations of resveratrol. In vitro release of resveratrol of different formula was measured using UV spectrophotometer. In vitro release patterns of all implants were assessed in phosphate buffered saline and Lipofundin. Morphological characteristics of the implants were examined using scanning electron microscopy (SEM). Balb/C mice were transplanted with EMT6/P cell line and in vivo antitumor activity was assessed for four groups: resveratrol injection treatment, treatment with PLGA implants loaded resveratrol, treatment with empty PLGA implants (vehicle), and untreated mice. Changes in tumor size were measured for each treatment. Histological examination of tumor sections was performed using standard hematoxylin/eosin staining protocol and TUNEL colorimetric assay was used to test the apoptosis induction ability for all treatments. ELISA was used to measure serum levels of interferon gamma (INF-?), IL-4, IL-2, and IL-10. Serum levels of the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were used as biomarkers of hepatotoxicity and serum creatinine was used to measure nephrotoxicity. Poly lactide co-glycolic acid implants loaded with 40% resveratrol released ideal concentrations of resveratrol compared with other formula, Glycerol Mono Stearate was the best enhancer added to PLGA implant to reach the best release. Implants were fully degraded within 14 days. RES implant caused a significant decrease in tumor size with a percentage cure of 80%. This therapy induced extensive necrosis and increased apoptosis in tumor sections. Serum levels of INF-? and IL-2 were increased in mice treated with resveratrol implants therapy. AST, ALT, and creatinine serum levels were close to their normal values. In Conclusion Our data indicate that PLGA implants loaded with resveratrol represent an active and safe option to treat breast cancer. The anticancer effect of resveratrol implants is mediated by induction of apoptosis, inhibition of cell division, and activation of T helper 1 anticancer immune response.

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(1) Assistant Professor, Clinical Laboratory Sciences, college of pharmacy, Iraq.
(2) , , University of Baghdad, Iraq.
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(5) , , vet.med., Iraq.

Abstract:
Cryptosporidiosis is an intestinal protozoan parasitic disease that infects human and animals, caused by apicomplexan parasite belong to the genus of Cryptosporidium. The current study was done to record the infection rate of cryptosporidiosis in human and cattle, and genotype the clinical isolates of Cryptosporidium in Baghdad Province. A total of 265 stool sample were collected (150 from human and 115 from cattle) during the period from December 2016 to the May 2017. Cryptosporidial infection was detected using modified acid fast stain. DNA of the parasite was extracted from oocysts of positive fecal samples and nested PCR method was used for partial 60 kDa glycoprotein (gp60) gene amplification then sequence analysis for selected samples. The total infection rates of Cryptosporidium in human and cattle were 47.33% (71/150), 35.63% (41/115) respectively. The results of this study record that Cryptosporidium parvum was found in all positive samples of human and cattle except two human samples which were Cryptosporidium hominis, and all were belonging to the common allele family IIa. The predominance of zoonotic subtype family of C. parvum species (IIa) in the present study highlights the significance of zoonotic transmission of cryptosporidiosis in the country. Keywords: Cryptosporidium, human, cattle, genotyping, Baghdad.

Reference:

Attachment:
Table1:
http://membs.org/membs/uploads/congress_speaker_files/1526950993Table 1.docx
Table2:
http://membs.org/membs/uploads/congress_speaker_files/1526950993Table 2.docx
Table3:
http://membs.org/membs/uploads/congress_speaker_files/1526950993Table 3.docx
Table 4:
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Figure 1:
http://membs.org/membs/uploads/congress_speaker_files/1526950993Fig.docx
Dihydrotestosterone regulates expression of CD44 via miR-328-3p in triple-negative breast cancer cells

Alothman, Nihad (1)

(1) Lecturer, Department of Anatomy, Biochemistry, and Genetics, Faculty of Medicine and Health Sciences, An Najah National University, Palestine.

Abstract:
Dihydrotestosterone regulates expression of CD44 via miR-328-3p in triple-negative breast cancer cells Nihad Al-Othaman1, Randa Bawadi2, Hana Hammad1, Maysa Al-Husseini3, Mamoun Ahram2 1School of Science, 2School of Medicine, The University of Jordan, Amman, Jordan 3King Hussein Cancer Center, Amman, Jordan Triple-negative breast cancer (TNBC) is an aggressive subtype that lacks effective targeted therapeutics and has poor prognosis. Targeting androgen receptor (AR) in TNBC is thought to be a promising approach. As a transcription factor, we hypothesized that AR controls cell behavior via regulating the expression of microRNA molecules (miRNAs). The expression of 84 breast cancer-specific miRNAs in MDA-MB-231 cells, a highly invasive TNBC model system, was investigated using PCR arrays following treatment of cells with 5α-dihydrotestosterone (DHT). Although these cells express AR at very low levels, it was sufficient to change the expression of 33 miRNAs by more than 2 folds including miR-328-3p, which was up-regulated by 14 folds. Transfection of cells with either miR-328-3p mimic or anti-sense decreased cell motility. As a target of miR-328-3p, DHT-mediated effect of expression and function of CD44 was investigated. The expression of CD44 and adhesion of cells to hyaluronic acid (HA) were reduced by treatment of cells with DHT or transfection with a miR-328-3p mimic. On the other hand, the AR antagonist, bicalutamide, or transfection of cells with miR-328-3p anti-sense had the opposite effect. Cells transfected with miR-328-3p anti-sense reduced the negative effect of DHT on CD44 expression and cell adhesion to HA. In addition, DHT further reduced the expression of CD44 and cell adhesion to HA in cells transfected with miR-328-3p mimic. At the tissue level, the expression levels of 7 miRNAs were found to be significantly associated with expression of AR in TNBC. Interestingly, 4 of these miRNAs had altered expression in MDA-MB-231 cells treated with DHT including miR-328-3p. These results strongly suggest that miRNAs can mediate AR regulation of breast cancer cells and that AR controls the expression of CD44 via miRNA-dependent and independent mechanisms.

Reference:
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Identification of potential druggable target against HCV NS3 genotype 3a

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(2) Ph.D research fellow, Center of Biotechnology and Microbiology University of Peshawar, Center of Biotechnology and Microbiology University of Peshawar, Pakistan.
(3) Assistant Professor, Centre of Biotechnology and Microbiology, Univeristy of Peshawar, Pakistan.
(4) Assistant Professor, Center of Biotechnology & Microbiology university of peshawar, University of peshawar, Pakistan.

Abstract:

Among the HCV non-structural proteins, NS3 has been a target for inhibition by numerous DAA (direct acting antiviral) drugs. The success rate for the treatment of HCV genotype 1 remained very high however treatment of genotype-3a, which is prevalent in Pakistani population, is still quite challenging. In a current study, homology models of HCV NS3 genotype-3a were generated using HCV NS3 genotype-1b as a template. Best protein model was then subjected to molecular docking. Finally, molecular dynamics simulation studies (MD simulation) were performed for Quercetin and Esculetin, against NS3 helicase domain of genotypes-3a. Our computational results show that Quercetin has successfully blocked the active site of helicase domain showing strong binding interaction between ligand and catalytic residues however the inhibitory potential of Esculetin on helicase domain of NS3 was not satisfactory due to weak binding interaction. These compounds may be subjected to lead optimization studies to further enhance their binding affinity. We conclude that Quercetin has a strong potential to be used as an effective anti-HCV drug. This study is a step ahead in the development of new potential druggable target against HCV-genotype 3a.

Reference:


Attachment:
Saima_Ikram_Flowchart_Poster:
Polymorphism of IL28B Gene (rs8099917) and Treatment Response in Pakistani Population

Ahmad, Fawad (1), Ikram, Saima (2), Ahmad, Jamshaid (3), Ur Rehman, Irshad (4)

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(2) Ph.D research fellow, Center of Biotechnology & Microbiology university of peshawar, University of peshawar, Pakistan.
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(4) Assistant Professor, Center of Biotechnology & Microbiology university of peshawar, University of peshawar, Pakistan.

Abstract:

Among liver disease Hepatitis C infection is one of the most widely spread infection resulting in hepatocellular carcinoma and liver cirrhosis. It is estimated that around 71 million people worldwide develop a chronic form of HCV infection and approximately 399,000 individuals die as a result of cirrhosis and hepatocellular carcinoma. Efficient antiviral therapy might prevent this lethal infection, but present treatment does not produce sustained virological response in chronic HCV patients. Pegylated interferon (PEG-IFN) in combination with ribavirin is commonly used therapy for HCV infection. Among majority of the patients treated with PEG-IFN and Ribavirin, only a few get a sustained virological response (SVR). HCV treatment method has altered significantly by the introduction of novel antiviral agents that provide better safety profile and improved antiviral potency. Combinations of these agents in IFN-free regimens are appropriate for chronic hepatitis C (CHC) infection.

On the other hand, host genetic factors also have vital role in natural clearance of HCV infection and induced treatment response in chronically infected patients. In this study, polymorphisms of IL28B gene (rs8099917) in AJK population were analyzed and their association with the virological response to treatment was determined. We found three types of genotype in rs8099917 of IL28B; wild-type TT in (38%) of patients, heterozygous GT minor genotype in (7%), and GG in (55%). We conclude that HCV-infected patients carrying homozygous GG have a higher chance of SVR as compared to those patients who carry G/T and T/T (rs8099917) which exhibit higher chances of RVR.

Reference:

The Use of Chick Chorioallantoic Membrane (CAM) as a Model to Study Engraftment of Human Breast Cancer Cells

Ali Deeb, Eiad (1)

(1) PhD student and lecturer, Department of Animal Production, School of Agriculture, Damascus University, Syria.

Abstract:

Chick embryo is considered as an attractive model to study engraftment and proliferation of human cancer cells due to being immunologically deficient. In this regard, chorioallantoic membrane (CAM) was previously used in few studies and proved to be efficient in transferring xenograft cells to many chick embryo organs, either through direct cell transplantation onto CAM or injecting cells into CAM veins. This study aims to evaluate the transfer of cells derived from a primary tumor of human breast cancer into chick embryo and to compare the transplantation and injection methods, extrapolating results from multiple embryonic organs. Indeed, results from conventional polymerase chain reaction (PCR) and reverse transcriptase PCR (RT-PCR) after injection into CAM vein showed the ability of human cell engraftment in either livers, hearts or lungs of chick embryos injected with 20-50 X 10^3 human cells, as specific bands appeared for PCR products on electrophoresis gels using primers specific for human GAPDH and FLT-3 genes, whereas no bands appeared in buffer-only injected embryos. In addition, a significant increase was found in weights of lungs of embryos injected with human cells in comparison with controls. In contrast, results from direct transplantation on CAM came negative as no specific amplification products showed up in cells derived from transplantation area. Here, we provide detailed protocols for direct transplantation and injection human cells via CAM membrane. The results of our study, the first in Syria, can pave the way to use chick embryo as an in vivo model to study the proliferation behavior of human cancer cells derived from solid or hematologic tumors, especially that this model is considered to be cheaper and easier compared to all other relevant animal models.

Reference:


Attachment:

Presentation:

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Modulatory effect of Capparis spinosa flower bud aqueous extract on edema and cytokines produced by peripheral blood mononuclear cells

Kernouf, Nassima (1), Bouriche, Hamama (2), Messaoudi, Dailila (3), KADA, Seoussen (4), Assaf, Areej (5), Senator, Abderrahmane (6)

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(3) Lecturer, Department of biochemistry, University Ferhat Abbas Setif 1, Algeria.
(4) Assistant lecturer, Department of biochemistry, University Setif 1, Algeria.
(5) Associate Professor, Faculty of pharmacy, University of Jordan, Jordan.
(6) Lecturer and Researcher, Department of biochemistry, university batna 1, Algeria.

Abstract:
In the present study, the anti-inflammatory activity of the aqueous extract of Capparis spinosa buds was evaluated in vivo by using paw edema inflammatory model. Moreover, the ability of the extract to modulate the release of TNF-?, IL-1?, IFN-? from peripheral blood mononuclear cells stimulated by concanavalin A was evaluated in vitro. Results showed that the oral administration of 200 and 400 mg/Kg of the extract decreased significantly (p < 0.05) carrageenan-induced paw edema. After 2 h, the inhibitions values were 47.62 % and 38.40 %, respectively. On the other hand, the treatment of the mononuclear cells with different concentrations (1, 10, 50 and 100 µg/mL) of the extract showed a significant (p < 0.001) inhibitory effect on the release on IFN-?. At 50 and 100 µg/ml, the inhibitions values were 32% and 53%, respectively. In contract, the treatment of cells with the same concentrations of the extract induced in dose dependant-manner the production of the TNF-? and IL-1?. Indeed, at 100 µg/ml the amounts of these cytokines were elevated by 4 and 3 fold, respectively. Taken together, we suggest that the aqueous extract of Capparis spinosa buds is effective as an anti-inflammatory agent by inhibiting edema and its action can be correlated with the inhibition of some inflammatory mediators including IFN-?.

Reference:
THE EFFECTS OF ERCC1 EXPRESSION LEVELS AND POLYMORPHISM ON THE CHEMOTHERAPY RESISTANCE OF BREAST CANCER PATIENTS TREATED WITH PLATINUM BASED ADJUVANT CHEMOTHERAPY

khalid, suhad (1), Ibraheem, Mohammed (2), Abbas Abdul-Majeed, Ban (3)

(1) Lecturer, Genetic Engineering, University of Baghdad, Iraq.
(2) Head of the Department, Genetic Engineering, Genetic Engineering and Biotechnology Institute / University of Baghdad., Iraq.
(3) Consultant, Molecular Pathology and Genetics, Al Nahrain University, Iraq.

Abstract:
Background and objective: The function of the excision repair cross-complementation group 1 gene ERCC1 impacts on the DNA repair process, particularly in anticancer therapy. The objective of this study is to assess the joint effect of ERCC1 mRNA expression and genotype on breast cancer patients’ response to platinum-based chemotherapy. Methodology: This is a case control study involving 44 breast cancer patients on chemotherapy and 31 apparently healthy women involved as a control. Genomic DNA and RNA were extracted from patients’ blood. ERCC1 and reference gene (β-actin) cDNA fragments were amplified by Real time qPCR and subjected to relative quantification. Genomic DNA was subjected to genotyping by Taqman Real time PCR to study the SNPs. Results: A significantly higher expression levels of ERCC1 was detected in patients than controls. Genotyping analysis revealed that patients carrying the C/T and T/T genotypes of rs 16115 of ERCC1 had higher gene expression whereas the ERCC1 C8092A rs3212986 SNP did not associate with breast cancer risk. Conclusion: The presence of mutant allele of ERCC1 at the rs16115 was associated with an increase mRNA level suggesting its possible effect on resistance to chemotherapy.

Reference:
Discovery of an ancestral mutation in US2A gene in an Algerian family

Samia, Abdi (1), Makrelouf, Mohamed (2)

(1) saad dahleb university, medecine, CHU Blida , Algeria.
(2) Director of Research Laboratory Biochemistry Genetics, CHU Bab El Oued - Université Alger 1, Algiers, CHU BAB-EL-OUED, Algeria.

Abstract:

Introduction: Deafness is the most common sensory deficit in children. Its social consequences depend on the moment of appearance and its severity. They mainly concern the communication and the acquisition of the language. It presents a genetic heterogeneity. The aim of this work is to investigate the genetic causes of this deficit in the Algerian population.

Materials and methods: Some fifty Algerian families with at least one case of neurosensory hearing loss are recruited at the central laboratory of Blida University Hospital. All the members of the families were taken on EDTA tube and the DNA is extracted by the « salting out » method in the laboratory of genetic biochemistry of CHU Babouled. The molecular study was done at the institute of vision in Paris. Results and discussion: Several mutations have been found among which this ancestral deletion C.2299delG in the exon 13 of the gene USH2A which was diagnosed in the homozygous state in a member of a consanguineous Algerian family. It is a mutation that is distinguished by its high frequency through several studies and has been described in several European and American families. But to date this deletion has not been described in North Africa. Conclusion: The genetic diagnosis of congenital deafness is essential for early management of sensory deficit and other deficits in the case of syndromic deafness. It also allows genetic counseling and avoids consanguineous marriages for members carrying heterozygous mutations.
Shisha microbiota - the good and the bad

Abdel Nour, Afif (1)

(1) Associate Professor, FAFS, USEK, Lebanon.

Abstract:

Over the last decade, there has been a rapid expansion of the trendy water pipe smoking around the world especially among younger adults. The initial objective of this study was to identify the microbiota of the shisha, which may either be of no harm for the smoker or enhance the threat on his well-being. The total DNA for the metagenomics study was conducted on three different shisha from three different delivery shops in Jounieh, Lebanon. The microbiota in two solid parts of the shisha, shaft & hose, were analysed including the fresh tobacco and the water in the bowl. All samples were analysed using high-throughput sequencing of 16S rRNA gene amplicons. Overall, more than 40 bacterial genera were found in the three investigated shishas, some are commensal others are pathogenic. All three shishas showed similar microbial content regarding the bacteria inhabiting in water, shaft, or hose. From the results of this study it appears that a very large amount of bacteria were found in the water pipes, some are harmful and others beneficial. We assume that the presence of gut dependent microbiota is related to the loose hygienic conditions in which the shisha is prepared.

Reference:

Identification of Disease Causing Mutations in Two Unrelated Jordanian Families with Cerebellar Ataxia.

(1) Lab supervisor, Cell Therapy Center, University of Jordan, Jordan.
(2) Assistant Researcher, Cell Therapy Centre, University of Jordan, Jordan.

Abstract:

Identification of Disease Causing Mutations in Two Unrelated Jordanian Families with Cerebellar Ataxia. Dema Ali, Ban Alkurdi, Bela Azab, Abdee Ryalat, Basil Sharrack, Abdalla Awidi, Nidaa Ababneh Background: Ataxia with oculomotor apraxia type 1 (AOA1) is a rare autosomal recessive disease, characterized by slowly progressive early-onset cerebellar ataxia, accompanied with oculomotor apraxia and a severe primary motor peripheral axonal motor neuropathy. The clinical phenotype of AOA1 is characterized by nystagmus, dysarthria, peripheral axonal neuropathy, muscle weakness, and distal loss of position and vibration sense (Bomont et al., 2000; Tachi et al., 2000). AOA1 is caused by mutations in the APTX gene. The APTX gene encodes for a nuclear histidine triad (HIT) protein, named aprataxin, involved in DNA single-strand break repair (Moreira et al., 2001b). Aims: (1) To detect the disease-causing variants in two unrelated families with cerebellar ataxias using whole exome sequencing (WES). (2) To correlate the clinical phenotype with the resulted APTX gene mutation identified by the WES. (3) To study the effect of APTX mutation on the protein and gene expression levels using blood samples and skin-derived fibroblasts. Methods: Whole-exome sequencing was performed to identify disease-causing variants in affected family members with hereditary autosomal-recessive form of cerebellar ataxia. Segregation analysis of candidate variants was assessed on affected and unaffected family members using Sanger sequencing. Functional analysis on cultured patients’ fibroblasts was carried out using reverse transcriptase polymerase chain reaction (RT-PCR) and western blot analysis, to study the effect of disease-causing variant on the protein and gene expression levels. Additionally, genotoxicity assays were also performed to detect the sensitivity of APTX mutated cells to genotoxic agents. In addition, Magnetic resonance imaging (MRI) and nerve conduction velocity (NCV) test were performed in all patients to assess nerve damage and dysfunction. Results: Whole-exome sequencing showed a recurrent homozygous nonsense variant in APTX (c.879G>A, p.Trp293Ter), consistent with the diagnosis of autosomal recessive AOA1. Segregation analysis showed that this variant completely co-segregated with disease phenotype in the entire tested families. Functional studies showed no detectable APTX protein in protein lysates obtained from cultured skin fibroblasts derived from patients. The clinical findings showed that all affected members have severe mixed axonal polyneuropathy, and cerebella atrophy as shown by MRI. More than 80% of the analyzed patients have hypoalbuminemia and hypercholesterolemia. All affected members show progressive ataxia and weak or loss of deep tendon reflex (DTR). These individuals are also dysarthric with hand athetosis and gaze palsy. Limb edema was also detected in some of the patients with walking difficulties, and complete areflexia. Conclusion: We report the phenotype variability across the AOA1 patients with nonsense variant in the APTX gene (c.879G>A; p.Trp293Ter). This study confirmed the importance of WES as a clinical tool to assist in the diagnosis of mendelian disorders.

Reference:

Knowledge, awareness and practices towards seasonal influenza and its vaccine: implications for future vaccination campaigns in Jordan

Abu-rish, Eman Y. (1)

(1) Associate Professor, department of biopharmaceutics and clinical pharmacy, school of pharmacy, The university of Jordan, Jordan.

Abstract:
Background. Influenza is an underestimated contributor to morbidity and mortality. Population knowledge regarding influenza and its vaccination has a key role in enhancing vaccination coverage. Objectives. This study aimed to identify the gaps of knowledge among Jordanian population towards influenza and its vaccine, and to identify the major determinants of accepting seasonal influenza vaccine in adults and children in Jordan. Methods. This was a cross-sectional study that enrolled 941 randomly selected adults in Amman, Jordan. A four-section questionnaire was used which included questions about the sociodemographic characteristics, knowledge about influenza and the factors that affect seasonal influenza vaccine acceptance and refusal. Results. Only 47.3% of the participants were considered knowledgeable. About half of the participants (51.9%) correctly identified the main influenza preventative measures. Lack of knowledge about the important role of seasonal influenza vaccine in disease prevention was observed. Low vaccination rate (20% of adults) was reported. The most critical barrier against vaccination in adults and children was the concern about the safety and the efficacy of the vaccine, while the most important predictors for future vaccination in adults and children were physician recommendation and government role. In children, the inclusion of the vaccine within the national immunization program was an important determinant of vaccine acceptance. Conclusion. Formulating new strategies to improve the population’s level of knowledge, assuring the population about the safety and the efficacy of the vaccine and the inclusion of the vaccine within the national immunization program are the essential factors to enhance vaccination coverage in Jordan.
Absorption of heavy metal by Microalgae in Red Sea, Saudi Arabia

Alamri, Aisha (1)

(1) Master Student, marine biology, King Abdulaziz University, Saudi Arabia.

Abstract:

Costal water of the red sea in Jeddah, Saudi Arabia exposed to a large proportion of pollution caused by industrial and economic development of the broad region known especially from desalination and sewage treatment. The potential environmental impacts resulting from the nutrient levels in the Red Sea environment has become a major concern because of the high toxicity. Microalgae have high ability to bind trace metals, where is considered an important indicator of the presence of pollutants in the sea environment. The use of living organisms such as micro-algae to identify areas of trace metals pollution and these organisms concentrate minerals from the surrounding water. In this study the samples will be collected of microalgae in the Red Sea then mixed micro-algae trawl-net samples taken from the water will be analyzed, which can provide information on the general characteristics of micro-algae nutrients and seasonal changes in nutrient absorption of micro-algae. We are expecting that minerals analysis of water samples showed broad similarities to laboratory cultured micro-algae in relation to nutrient absorption. This can be used as water treatment technique.

Reference:

Effect of He-Ne Laser on the Lymphocyte Cells and their DNA

Sabah, Siham (1), Hussein Taha, Jenan (2)

(1) Al-Nahrain University, Department of Physiology & Medical Physics, College of Medicine, Iraq.
(2) Al-Nahrain University, Department of Physiology & Medical Physics, ministry of higher education, Iraq.

Abstract:

Background Light laser is widely used for a wide range of medical applications. He-Ne laser application in medicine as in any type of laser is based on the interaction of laser light with the biological system. Objective The present study was done to show the effect of He-Ne laser (632.8 nm) irradiation on human lymphocyte blood cells and their DNA. Method This study involved 72 blood samples, taken from healthy volunteers. The samples were divided into two groups. The first group consisted of 27 samples were processed only for lymphocyte blood cells separation, while the second group, which consisted of 45 samples were employed to evaluate the influence of He-Ne laser irradiation on the extracted DNA from the lymphocyte blood cells. Results At the used doses of He-Ne laser (18, 35, 52.5, and 69 J/m²), a significant difference was found (P < 0.05) in survival percentage of lymphocyte cells (99.8, 99.74, 99.68, and 99.59) in comparison with those cells untreated with He-Ne laser irradiation. Immediately after He-Ne laser irradiation alone, the following doses (18, 35, and 69 J/m²) were applied on the extracted DNA, the DNA demonstrated a significant damaging where the fraction of DNA survival percentage was (88.6, 87.7, 86.1) respectively, with significant difference (P < 0.05) between the DNA survival before and after He-Ne laser irradiation. Conclusion In conclusion, the percentage of lymphocytes survival is decreasing with increasing dose of He-Ne laser and longer exposure time where time exposure (2.5, 5, 7.5, and 10 s). He-Ne laser irradiation causes a significant degree of DNA damaging independent on the irradiation doses. Keyword Lymphocyte cells, He-Ne laser irradiation, DNA

Reference:

Ataxia Telangiectasia Mutated Serine Threonine Protein Kinase, the linchpin support of antineoplastic platform.

Hussain, Tahir (1), Hussain, Tahir (2)

(1) Lecturer, Biological Sciences, WISE College, Pakistan.
(2) Lecturer, Science, WISE College, Pakistan.

Abstract:

ATM a DNA DSBs damage sensor plays pivotal role in cell cycle checkpoints. Certain Genotoxic stresses / irradiation instigate towards its activation. Any mutation in ATM infringes cell cycle restraints thence orchestrating towards neoplastic proliferation of cells which is a distinctive feature of cancer. Since its appraisal as a disease related entity much has been deciphered vis-à-vis genomic instability, cancer and other related pathologies. More than 700 putative substrates bind ATM / ATR to regulate host of cellular functions. It adds to its dismay when claims of about 432 different mutations in ATM are being positively validated and unswervingly implicated in cancer predisposition. ATM tends to recapitulate the normal dynamics of a cell. If ATM is aberrant, the cellular restraints are hampered. In such scenarios when cellular stability is compromised, the cell may be sequestered for perpetual proliferation or it may be orchestrated towards apoptosis mediated by several different key player molecules. Ataxia Telangiectasia mutations ensue autosomal recessive pattern of inheritance. ATM related conditions are a paradigm for cancer predisposition and related pathologies. It underscores the need for exploration of its gene processing and regulatory elements as well as PTMs for phenotypic effects.

Reference:


Effect of Alo vera gell extract on blood glucose level and lipid profile in induces diabetic moue

Mohammed Hasan, Zainab Yaseen (1)

(1) Head Researcher, Biotechnology Research Center, Al Nahrain University, Iraq.

Abstract:

Abstract: Hyper glycemia and hyperlipidemia are both getting epidemic property in our country, that make an important to search a medicinal plant extract to solve elevated cases. Alo vera has been cultivated in Iraq, rich with many active constituents. This study emphasized on the alcoholic gell extract to treat diabetic induced mice with streptozotocin, and the mice were fed on high fatty food to elevate lipid profile before treating with Alo gel extract. Results showed that oral administration A. vera extract and the anti diabetic drug Glibenclamide in a dose of 300mg/kg, 600µg/kg body weight respectively; the glucose level had been decreased after one week with both treatment. After Three weeks later blood glucose level was at lowest level with A. vera fed mice. Effect of A. vera extract on lipid profile including level of cholesterol (Chol.), triglycerides (T.G) and High density lipoprotein (HDL); after 21 days from the plant extract and the drug treatment, there was a decrease in cholesterol level and in T.G level, even in normal mice fed with the extract only. HDL level showed no change for the extract treatment than the diabetic negative control.
Investigation of Esco1/2 function and regulation during sister chromatid

Fakhurji, Burhan (1)

(1) Senior Specialist (Virologist & Molecular Biologist), Medical Laboratory, Ministry of Health, Saudi Arabia.

Abstract:

Cohesin of sister chromatid is controlled by a multi protein cohesin complex, which is composed of Smc1, smc3, Scc1 and Scc3 (SA1 and SA2 in vertebrate). In mammalian cells, chromosome cohesin depends on acetylation of Smc3. Esco 1 and Esco 2 proteins have acetyltransferase and C2H2 zinc finger domains at N-terminus, which allows them to play an important role in Smc3 acetylation. During the S phase of the cell cycle, Esco 1 and Esco 2 contribute to the establishment of sister chromatid cohesion. Esco 1 plays a key role in DNA repair and cell survival (Gordillo et al., 2008). Nevertheless, both proteins are important for stabilisation of chromosome cohesin and may also have an important role in human congenital syndromes such as Roberts Syndrome (RBS). Previous work has shown a mobility shift in Esco 2 on SDS-PAGE gels. This result suggested that Esco 2 may be post-translational modification. The aim of this study was to investigate the regulation of Esco 1/Esco 2 and how they targeted to the chromatin in order to acetylate of Smc3. My results indicate that Esco 2 protein levels are controlled by posttranslational modification during mitosis, possibly by ubiquitination. Conclusions I attempted to investigate the regulation of Esco 1 and Esco 2 during the cell cycle. The two proteins Esco 1 and Esco 2 were depleted in HeLa cell using siRNA. The antibody to Esco 1 was not very specific, tend second antibody but do not know if it binds to Esco 1 as no depletion of Esco 1 protein was observed after Esco 1 siRNA. According to the results of the Western blot analysis, it seems that the Esco 2 protein levels are controlled by posttranslational modification possibly ubiquitination. To prove this, we will overexpress Flag-Esco 2 with HA-ubiquitin. We will immunoprecipitate Esco 2 and Western blot with HA antibody to see if Esco 2 is ubiquinated. Finally, if we can overexpress Esco 1 then we will be able to immunoprecipitate it and examine its phosphorylation state by mass spectrometry. Further study There are several questions need to be investigated, it would be interesting to examine whether the independent replicated Smc3 acetylation contributed to regulating the DNA transcription. Also, the primary functions of Esco 1 of the mammalian cells, as much as the acetylation is curial essential for extracting cells. Note: This paper is the project of a master’s degree from The University of Leicester All rights reserved

Reference:

Attachment:
Jordon 2018:

CV:
Generation and Characterization of Induced Pluripotent Stem Cells (iPSCs) from Patients with Ataxia Oculomotor Apraxia Type I

Alkurdi, Ban (1), Anwar Ababneh, Nidaa (2)
(1) Researcher, Cell Therapy Center, Jordan University, Jordan.
(2) Assistant Researcher, Cell Therapy Centre, University of Jordan, Jordan.

Abstract:
Ataxia oculomotor apraxia type 1 (AOA1) is an autosomal recessive neurological disorder associated with mutations in the Aprataxin gene (APTX), located on chromosome 9p13. Physical features of the disease include cerebellar ataxia, oculomotor apraxia, peripheral neuropathy and its often associated with hypoalbuminemia and hyperlipidemia. Degeneration of cerebellar Purkinje cells responsible for movement coordination is the most common feature among AOA1 patients. The encoded Aprataxin protein interacts with X-ray repair cross-complementing protein 1 and 2 (XRCC1/2) along with other proteins, forming multi-protein complexes involved in single and double strand DNA repair (SSBR and DSBR). Since APTX is a major component of the DNA repair machinery mutations in this protein are predicted to influence cellular response to genotoxic stressors. However, the exact underlying disease pathophysiology is yet to be uncovered. Induced pluripotent stem cells (iPSCs) represent a powerful tool that have been utilized for years in disease modeling, drug screening and currently undergoing clinical studies as a potential source for autologous cells that could be used in tissue engineering and cell replacement therapy. The use of iPSCs in disease modeling is of unique importance as it opens the doors for the analysis of disease pathophysiology in cells and tissues of inaccessible origins most importantly nerve and cardiac tissues. The concurrent use of iPSC with genome editing technologies such as clustered regularly interspaced short palindromic repeat (CRISPR) system allows for the correction of mutant alleles in affected cells, as well as introducing such mutants in wild type cells. The combination of these two techniques enhances our understanding of disease pathways by generating isogenic pairs, allowing for high-throughput personalized drug screening and potentially for personalized gene therapy. Previously, we performed whole exome sequencing (WES) on DNA samples from three affected subjects of two unrelated families. WES results revealed the presence of c.879 G>A mutation in the APTX gene. The aim of our study is to analyze the effect of this mutation on protein expression and to generate and characterize iPSC lines from AOA1 patients carrying the APTX mutation to study the disease phenotypes. Skin biopsies were utilized to derive patient’s specific fibroblasts. RNA and protein extraction were carried out on the derived cells. Quantitative polymerase chain reaction (qPCR) and western blot were performed to analyze the expression of APTX at the RNA and protein level. Gene expression analysis revealed no significant difference in APTX mRNA expression between controls and patients. However, western blot analysis revealed that c.879 G>A results in protein termination which interferes with protein translation, as depicted by the lack of APTX protein in cells of affected individuals. Additionally, it has been reported that mutations in APTX gene are associated with increased susceptibility to genotoxic stress. To study this response in APTX deficient cells, genotoxic agents such as methyl methane sulfonate (MMS), mitomycin C (MMC), H2O2 and epotoside were applied on AOA1 derived fibroblasts. To generate patient specific iPSC lines, fibroblasts were transduced with CytoTune™ 2.0 Sendai reprogramming vectors carrying the four Yamanaka reprogramming factors. Established iPSC lines were characterized to check for their ability to express the pluripotency markers OCT-3/4, SOX-2, SSEA-4 and TRA-1-60 by flow cytometry and immuno-cytochemistry. Sendai virus clearance by qPCR was performed to ensure the complete removal of the RNA vector without any integration to the host cells. Finally, cells were expanded in feeder free environment and these cultures were utilized for neuronal differentiation. We further plan to differentiate patient specific iPSCs derived cells into motor neurons and assess the effect of different genotoxic agents on these cells. Additionally, we are aiming for the generation of isogenic lines from cells of affected individuals using CRISPR-Cas9 system. The generation of these lines will open the doors for high-throughput drug screening, potential gene therapy and cell replacement therapy in the near future.

Reference:
The applications of Molecular Biology in the Clinical Nutrition practice

Labban, Louay (1)

(1) Head of Nutrition and Dietetics, Nutrition and Dietetics, TeSham Academy, Syria.

Abstract:
Molecular biology is the field of biology that study the composition, structure, and interactions of cellular molecules and nucleic acids proteins that carry out the biological processes essential for the cell’s functions and maintenance. The advances in molecular biology have substantially contributed to the understanding of how genes influence physiologic processes and, ultimately, our health. Food science and nutrition research have benefited immensely from such developments. Recent progress in this field has had a considerable effect on both basic and applied clinical nutrition and offers even greater potential for the future. Genes associated with many nutrition-related chronic diseases are being identified and characterized. Nutrients may directly or indirectly influence the transcription and/or translation of specific gene products. Identifying genetic markers for specific diseases and exploring gene therapy will provide new opportunities and challenges for clinical nutrition practice in coming days. Unraveling the multitude of nutrigenomic, proteomic, and metabolomic patterns that arise from the ingestion of foods or their bioactive components will not be simple but is likely to provide insights into a tailored approach to diet and health. The use of new and innovative technologies, such as microarrays, RNA interference, and nanotechnologies, will provide needed insights into molecular targets for specific bioactive food components and how they harmonize to influence individual phenotypes. Nutritional genomics can play an important role in the future in enriching foods with specific bioactive nutrients, developing functional foods and supplements and expanding the role of the dietitian to include genetic counseling and bioethics training.

Reference:
Explores the antibacterial potential of Lantana camara L. extracts collected from in vivo and vitro grown plant material

Shibli, Rida (1)

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Abstract:

Lantana camara L. is a well-known ornamental evergreen plant. Meanwhile, this flowering shrub is considered as medicinal plant and was used in traditional medicine for treatment of chicken pox, measles, asthma, ulcers and many more. Many researchers reported that extracts from the Lantana camara aerial parts were found to possess antimicrobial and antitumor activities, but most of the extracts used in their experiments were obtained from aerial parts of in vivo grown shrubs. Our study aimed to explore the antibacterial potential of Lantana camara L against selected strains of bacteria using extracts collected from in vitro grown plant material (microshoots and callus cultures) and to compare the results with those obtained from extract collected from aerial parts of in vivo grown plants using Disk diffusion and Microdilution assays. Data from Disk diffusion assay showed that, all types of Lantana camara L extracts possessed antibacterial potential against most types of the tested bacteria strains, but was most promising in vivo plants extract. For example, in vivo plants extracts resulted in growth inhibition zone diameter of (20 mm) in Bacillus subtilis compared to (12 mm) obtained in extracts of in vitro grown plant material. Meanwhile, antibacterial efficacy of the in vivo grown plant extract was very promising against Staphylococcus aureus and Enterobacter aerogenes, as it resulted in inhibition zones of (37, 28 mm; respectively), which were even higher than the antibiotic results. Moreover, extracts from in vivo grown plant material and microshoots acted similarly against Micrococcus luteus, Staphylococcus epidermidis and Klebsiella pneumoniae as they resulted in growth inhibition zone diameters of (27, 24, 23 mm; respectively) and were similar to values recorded by the antibiotic. Meanwhile, growth of Erwinia carotovora was not affected by extracts of in vitro grown cultures. In Microdilution assay, extracts from in vivo grown plant material were most effective against Bacillus subtilis, Staphylococcus epidermidis, Klebsiella pneumoniae, Salmonella sp. and Erwinia carotovora compared to the other extracts, while the three extract types resulted in full growth inhibition in Micrococcus luteus, Enterobacter aerogenes and E. coli at MIC values of (6250, 3125, 1562.5 µg/ml; respectively).
Investigating Antibacterial Potential of Ethanolic and Methanolic Extracts of Schinus molle L Tree

Tahtamouni, Reham (1)

(1) Assistant Professor, Department of Applied Science, Princess Alia University College, Al- Balqa Applied University, Amman, Jordan, Al- Balqa Applied University, Amman, Jordan, Jordan.

Abstract:

Schinus molle L. is an ornamental plant growing in Jordanian environment, was acknowledged recently for its therapeutic properties against many microbes. This study aimed to find out antibacterial potential of ethanolic and methanolic extracts obtained from different parts of Schinus molle L. tree against four strains of bacteria. Obtained data showed that, ethanolic and methanolic extracts from all experimented plant parts inhibited growth of Bacillus subtilis successfully. However, best results were obtained in ethanolic extract of leaves as it resulted in growth inhibition zone of (22.0 ±0.06 mm), while growth was completely inhibited at MIC value of (1.563 mg/ml). Moreover, growth of Enterobacter aerogenes and Klebsiella pneumoniae were mostly inhibited after exposure to methanolic extract of leaves it as resulted in inhibition zone of (18.0 ±0.086 and 17.0±0.12 mm) respectively, while full growth inhibition was obtained at MIC of (1.563 mg/ml). Meanwhile, results of disc diffusion assay indicated that Micrococcus luteus growth was slightly affected by leaves ethanolic extract, while all other types of either ethanolic or methanolic extracts failed to inhibit growth of this bacteria. However, results of microdilution assay revealed full growth inhibition of Micrococcus luteus when exposed to either fruit or leaves ethanolic extracts at level of (6.25 mg/ml), while methanolic extracts from all invistigated plant parts faild to prevent growth of Micrococcus luteus.
Evaluation of BAX, BCL-2 and Caspase3 genes in pathogenesis of endometriosis

Mohammadi, Roudabeh (1), Mousavi, Seyed Omidreza (2), Aghajanpour, Samaneh (3), Ramazanali, Fariba (4), Moini, Ashraf (5), Aflatoonian, Reza (6)

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(6) Head of the Molecular Biology Lab, Reproductive & Biomedicine, Royan Institute, Iran.

Abstract:

Background Endometriosis is characterized by the presence of endometrial cells with capacity to avoid apoptosis outside the uterus. Although the exact etiology of endometriosis remain unclear, recent studies show that apoptosis plays a fundamental role in the pathogenesis of endometriosis. Apoptosis is regulated by several genes, especially those of the Bcl2 gene family and caspase group genes. Different expression of these genes could contribute to the survival of endometrial cells into the peritoneal cavity and the development of endometriosis. This study was evaluated the different expression of BAX, BCL-2 and caspase 3 in endometrial tissue and endometriosis lesion. Materials and method To analyze the expression of BAX, BCL-2 and caspase 3 in endometrial tissues, we used ectopic and eutopic lesions from patients with endometriosis. The biopsies were divided into three groups: menstrual, proliferative, and secretory phases. Then compared them with normal endometrium during the menstrual cycle. Reverse Transcriptase PCR (RT-PCR) was performed on the prepared cDNA samples and using primers for the genes of interest in this investigation. Result Different expression of BAX, BCL-2 and caspase3 was indicated in samples with endometriosis and normal endometrial tissue. Conclusion Apoptotic genes expression were different in eutopic and ectopic endometriosis in compared to control. Expression of apoptotic genes in endometriosis was lower than control, which might effects on the growth and survival of ectopic endometrial tissue. Key word: BAX, BCL2, Caspase3, Endometriosis
The role of Tumor Necrosis Factor (TNF-?) and Estrogen Receptors genes (ER?, ?) in women with Endometriosis

golkar, sima (1), fallahi, salehe (2), aghajanpour, Samaneh (3), Ramazanali, Fariba (4), Moini, Ashraf (5), Aflatoonian, Reza (6)

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Abstract:

BACKGRAND: Endometriosis is a disease characterized by the development of endometrial tissue outside the uterus. Numerous genes have been studied and proposed to help explain its pathogenesis. It is one of the most widespread gynecological diseases with a 10-15% prevalence in the general female population, rising up to 30-45% in patients with infertility. Tumor necrosis factor alpha (TNF?) is a cell signaling protein (cytokine) involved in systemic inflammation and it is one of the cytokines that makes up the reaction. There are two different forms of the estrogen receptor(ER? and ER?). Estrogen receptors play a unique role in endometrial tissue, where they interact with the cytoplasmic apoptotic machinery and inflammasome complex to prevent TNF?-induced cell death and enhance adhesion and proliferative activities of endometrial tissues. Our aim in this study is to determine the expression of TNF? and ER?, ER? in female with endometriosis. MATERIAL AND METHODS: The expression of TNF?, ER?, ER? and their effects in eutopic and ectopic endometrial tissue which was obtained during standard surgery of women in reproductive age has been detected by Reverse transcriptase-polymerase chain reaction (RT-PCR). RESULT: The expression of TNF? and ER?, ER? were altered between normal and endometriosis groups in various phases of menstruation. CONCLUSION: We characterize differential expression of ER?, ER? and TNF? in endometriosis patients, and show molecular distinction of eutopic and ectopic endometrium of patients compared with control women. KEYWORDS: Apoptosis, ER?, ER?, Endometriosis, TNF?
The Role of Tight Junction Genes Expression in Cell Mobility of Endometriosis

taleb, shaghayegh (1), zhaeentan, shahrzad (2), aghajanpour, Samaneh (3), Ramazanali, Fariba (4), Moini, Ashraf (5), Aflatoonian, Reza (6)

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Abstract:

Background: Endometriosis is a condition in which the endometrium, the layer of tissue that normally covers the inside of the uterus, grows outside of it. Tight junctions are regulated in their molecular composition ultrastructure, and function by intracellular scaffolding proteins and the cytoskeleton; such regulation serves normal, physiologic adaptation but also occurs in numerous diseases. However, become increasingly apparent that the tight junction has a vital role in maintaining cell to cell integrity and that the loss of cohesion of the structure can lead to endometriosis. The aim of the study was to assess tight junction genes functions in the context of endometrial biology Material and methods: This research is a genomic study of tight junction genes claudin-3 and claudin-4 in eutopic and ectopic endometrial tissue specimens obtained during standard surgery of women in reproductive age by using RT- PCR. Results: The candidate gene expression altered in ectopic endometrial tissue specimens in compare with control group. Conclusion: We describe a genetic basis for endometriosis and provide strong evidence for the existence of the role of tight junction abnormality in endometriosis patients. Key words: Endometriosis, Ectopic, Eutopic, claudin-3, claudin-4, Tight junction
Evaluation of interleukin 6, 8 and STAT3 genes expression in patients with endometriosis

fallahi, salehe (1), golkar, sima (2), aghajanpour, Samaneh (3), Ramazanali, Fariba (4), Moini, Ashraf (5), Aflatoonian, Reza (6)

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(6) Head of the Molecular Biology Lab, Reproductive & Biomedicine, Royan Institute, Iran.

Abstract:

BACK GROUND: Endometriosis is a common benign disease of Gynecology, which often causes symptoms of dysmenorrheal, pelvic pain and infertility. The etiology and pathogenesis remain unclear. Cytokines, such as interleukin (IL)-6 and 8 are important factors involved in inflammation associated with endometriosis. STAT family carries out a dual function: signal transduction and activation of transcription. A new family member, Stat3, in response to IL-6 becomes activated and occupies the endogenous IL8 promoter which directly represses its transcription. The activation of STAT3 signaling plays an important role in the pathogenesis of endometriosis. Therefore we decided to evaluate the gene expression of IL6 and 8 and STAT3 in ectopic and eutopic endometriosis tissue of female with and without endometriosis. MATERIAL AND METHODS: With the RT-PCR using , we researched the expression of IL6, IL8 and STAT3 as reliable genes in ectopic endometrial tissue obtained from women of reproductive age. RESULT: During this study, changes in the expression of this gene were observed in the ectopic tissue of patients with and without endometriosis. CONCLUSION: Our result indicated that IL6, IL8 and STAT3 were associated with susceptibility to endometriosis. KEY WORDS: Endometriosis; IL6; IL8; STAT3.
Different expression of heat-shock protein 70, Toll-like receptor 2, 4 and Interleukin 10 between endometriosis and normal endometrium

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Abstract:

Background: Endometriosis is a chronic inflammatory condition that mainly affects women of reproductive age. Although the immunological mechanism of endometriosis is still unclear, the major theory of its pathogenesis is retrograde menstruation. Recent studies show that the growth of endometriosis can be regulated by the innate immune system in the pelvic environment. Toll-like receptors (TLRs) are a class of proteins which play a key role in the innate immune system and past studies have shown that TLRs particularly TLR2 and TLR4 are expressed in the endometriosis tissues. Tissue invasion in endometriosis may cause the release of endogenous heat-shock proteins (HSPs) into the pelvic environment. HSP70 is an intracellular protein which be recognized by toll-like receptors and can express IL10, an anti-inflammatory cytokine that regulates inflammation. Purpose: The objective of this study is to evaluate the expression of HSP70, TLR2, 4 and IL10 in endometriosis tissues and compare them with normal endometrium. Material and methods: Ectopic and eutopic tissues of endometriosis patients in different phases of menstrual cycle were collected and reverse transcriptase polymerase chain reaction (RT-PCR) was performed on the prepared cDNA samples to measure the gene expression levels of HSP70, TLR2, 4 and IL10. Results: Different expression of the genes was indicated in various phases of menstruation in eutopic and ectopic lesions. Conclusion: This study has shown that alteration in the expression of HSP70, TLR2, 4 and IL10 through TLR signaling pathway might have an important role in endometriosis symptoms. Our findings also suggest that IL-10 may suppress immunity against endometrial implants, contributing to development of endometriosis, therefore evaluation of the expression of these genes in endometriosis patients is highly recommended. Key words: Endometriosis, HSP70, IL10, TLR2, TLR4
Anti-inflammatory effects of Algerian Cistus salvifolius methanolic extract

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(4) Lecturer and Researcher, Department of biochemistry, university batna 1, Algeria.

Abstract:
Cistus salvifolius are wide spread in the north western Africa, used as general remedies in folk medicine for treatment of various skin diseases and inflammation disorders [1]. The aim of this study is to evaluate the anti-inflammatory activity of methanolic extract from the leaves of Cistus salvifolius using carrageenan-induced paw edema in Wistar albino rats [2] and air pouch model in Swiss albino mice [3]. Moreover, the release of IL-1? and TNF-? from concanavalin A-stimulated monocytes was examined[4]. Results showed that Cistus salvifolius methanolic extract, at 200 and 400 mg/Kg, exerted significant anti-inflammatory activity. Both doses reduced the paw edema by 67% and 86%, respectively. Similarly, the treatment of mice with 1 mg/pouch of the extract decreased the number of leucocytes migrated in the air pouch by 48.42%. This inhibition is statistically comparable to that of indomethacin, used as standard anti-inflammatory agent. Indeed, IL-1? release was reduced by 95% by the treatment with different concentrations (1, 10, 50 and 100 µg/ml) of the extract, while TNF-? release was reduced by 62% and 100% with 50 and 100 µg/ml of extract, respectively. The results obtained in this study revealed the anti-inflammatory activity of methanolic Cistus salvifolius extract justifying its use in folk medicine. Key words: Cistus salvifolius, anti-inflammatory, Paw edema, Air pouch, Cytokines

Reference:
Evaluation of Adherence Junction Genes in Endometriosis

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Abstract:

Background: Endometriosis is a condition in which the endometrial tissue grows outside of uterine cavity invasively. The interactions and connections between cells are important for the assembly and maintaining contiguity of epithelial layers. The adherens junctions are formed by two sub-complexes. The first one is nectin-based adhesion, which forms the first attachment of cells to their neighbors while the second is cadherin-based which mediates strong cell-cell adhesion. The most important type of cadherin-based adhesions is E-cadherin which mediates the adhesion of cells to adjacent cells, thereby preventing the separation of cells. Adherence junctions regulated the ultrastructure and function of cells; such regulation serves normal and physiological adaptation but the abnormal functions and structure causes numerous diseases. As the adherence junctions have vital role in maintaining cell to cell integrity in the case of decreasing the adhesion molecules it can lead to endometriosis. In the present study the adherence junctional genes expression detected in eutopic and ectopic endometrial tissues by RT-PCR. Material and Methods: The genes expression of adherence junctional molecules was investigated by RT-PCR in both ectopic and eutopic. The candidate genes were zona occludin-1 (ZO-1), Desmoglein-1 and E-cadherin. Results: The candidate gene expression have been decreased in ectopic tissue in compared with control group. Conclusion: We describe a genetic basis for endometriosis and provide strong evidence for the existence of the role of adherence junction abnormality in endometriosis patients. Key words: Endometriosis, cell junction, E-cadherin, Desmoglein-1, Zona occludin-1
Genetic diversity among Egyptian wheat cultivars using SCoT and ISSR markers

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Abstract:
ISSR and SCoT markers were used to investigate the genetic diversity and relationships among eight cultivars of Egyptian wheat (Shandweel 1, Misr 2, Sakha 93, Sakha 94, Giza 168, Giza 171, Sids 1 and Gemmiza 9). SCoT primers produced a total of 32 bands, out of which 19 (59%) were polymorphic with a mean of 3.16. ISSR primers produced 34 bands and 23 of these bands (68%) were polymorphic with a mean of 4.6. Moreover, PCR based specific primers was used for detection of P5CS gene in the wheat cultivars. These results indicated good sources of diversity which will help breeders to evaluate genetic diversity and potentially select economically important traits such as salinity tolerance.

Reference:

Attachment:
Wheat Biodiversity:
http://membs.org/membs/uploads/congress_speaker_files/1527613024Genetic diversity among Egyptian wheat cultivars using SCoT and ISSR markers.docx
Glucose Deprivation Enhances the Antiproliferative Effects of Oral Hypoglycemic Biguanides in Different Molecular Subtypes of Breast Cancer: An in vitro Study

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Abstract:
Recently, extensive in vitro studies have been conducted to evaluate anticancer activity of oral hypoglycemic agents. Many of these studies experienced detrimental limitations as they were conducted on cancer cells commonly grown in culture media consisting of extremely high concentrations of growth factors and glucose. The present study aimed to explore antiproliferative effects of the commonly studied metformin and the less frequently reported phenformin oral hypoglycemic agents on different molecular subtypes of breast cancer under rich glucose and glucose deprived conditions. Our results indicate that under glucose deprived conditions, which better reflect the factual glucose-starved solid tumors in vivo, biguanides demonstrate more antiproliferative activities against the three molecular subtypes of breast cancer cell lines examined in this study. In addition, the observed antiproliferative activities of biguanides appear to be mediated by apoptosis induction in breast cancer cells and this induction is significantly augmented under glucose deprived conditions.
Anticancer Activities of Crude Extracts of Alhagi graecorum on Two Cell Lines, RD and L20B in vitro

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Abstract:

The present study was designed to evaluate the anticancer effect of Alhagi graecorum (aqueous, ethanolic and hexane extracts) in vitro on two cell lines (RD and L20B) using different concentrations of Alhagi graecorum extracts (5, 10, 20, 40, 80, 160, 320 and 640 µg/ml) for an incubation period of 48 hours. The results revealed a clear cytotoxic activity of those extracts on growth of L20B and RD cancer cell line, and the effect was concentration-dependent. The results also, suggested that the ethanolic and hexane extracts of Alhagi graecorum showed the best cytotoxic activity on L20B and RD cell line, especially at the concentrations 320 and 640 µg/ml. In contrast, there was no significant cytotoxic effect of the aqueous extract on the L20B and RD transformed cells, with the exception of aqueous extract at high concentrations, in which a significant growth inhibitory effect was observed.

Reference:

Assessment of JAK2V617F Mutation and Serum Levels of Alkaline Phosphatase and Lactate Dehydrogenase in Myeloproliferative Neoplasm

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Abstract:
Myeloproliferative neoplasms (MPNs) are a clonal excess hematopoiesis stem cell and this disease characterized by proliferation of one or most of the myeloid lineages in the bone marrow. There are three main entities are polycythaemia Vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Total of (51) patients with suspicious myeloproliferative neoplasms (MPNs) and 20 healthy individuals analysed for the JAK2 V617F mutation, the mutation was detected by using (ARMS) PCR Amplification, activity of Alkaline Phosphatase and Lactate Dehydrogenase was measured spectrophotometrically. Out of 51 patients, the JAK2 V617F mutation (V617F) was detected in 33 out of 42, with PV (81%), and four of patients with ET and PMF (40% and 50%, respectively). The prevalence of this mutation is more associated with male than female about (62%). Our study results showed ALP activity which is significantly differences (p< 0.05), While LDH showed a highly significant level (P<0.01) in suspected MPN patients when compared to the control group. Aims of the present work are contain; Estimate the significance of Alkaline Phosphatase parameter and Lactate Dehydrogenase and relate them with mutation in MPN Iraqi patients, estimate the proportion of JAK2 V617F mutant gene in MPN Iraqi patients

Reference:
VKORC1 Variants as Significant Predictors of Warfarin Dose in Emiratis.

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(6) Professor, Department of Pharmacy, University of Patras, Greece.
(7) Professor of Molecular and Genetic Medicine, Department of Pathology, College of Medicine d Health Sciences, UAE University, United Arab Emirates.

Abstract:

Background: Vitamin K epoxide reductase complex (VKORC) is known to be the target enzyme of warfarin [1]. Numerous studies have established the contribution of VKORC1 gene variants on warfarin dose in patients from different populations. Some single nucleotide polymorphisms (SNPs) in VKORC1 were found to be common in diverse populations, while others were found sporadically and are considered rare [2]. In general, VKORC1 variants can explain approximately 20% to 30% of the variation in warfarin dosing [3]. Haplotype maps were established from the common VKORC1 SNPs. These efforts yielded two main ways to report VKORC1 haplotypes: the H system [4], and the star system [5]. Both systems were built in European populations. The star system relies on the genotypes at five variants (rs9923231, rs17708472, rs9934438, rs2359612, rs72924). In this system, *2 haplotype is associated with low dose warfarin while *3 and *4 alleles are associated with a high dose of warfarin [5]. Aim: The current study aimed at evaluating the effect of eight SNPs in VKORC1 on warfarin maintenance dose in a group of Emirati patients receiving a stabilized dose of warfarin. The chosen variants included those in the star system (five SNPs) and three more SNPs that were found to be actionable in some populations [6]. Furthermore, we aimed at estimating the frequencies of haplotypes and analyzing their association with warfarin dose. Methods: The study cohort included 90 unrelated Emiratis who have been treated with warfarin. Patients were recruited from the INR clinic at Tawam Hospital, Al-Ain, UAE. DNA was extracted from peripheral blood that was collected on EDTA. Sanger sequencing of almost the whole VKORC1 gene and its promoter was used for genotyping. Results: A significant difference (P=0.05) in average doses of warfarin among different genotype groups was found at the following SNPs: rs9923231, rs9934438, rs8050894, rs2359612, and rs7294. A difference was also observed between genotype groups at rs61742245 but at lower significance. A global test for statistical difference among star system haplotypes did not give a significant association with dose in linear regression analysis (P=0.62). In contrast, haplotypes extracted from the six significant variants in our population (rs9923231, rs61742245, rs9934438, rs8050894, rs2359612, rs7294) have shown significant global haplotype association with warfarin dose (P=0.009) (table1). A stepwise multivariate regression that included all the selected variants at VKORC1 with age and gender revealed that the main predictors for warfarin dose were rs9923231, age, and rs61742245. The most potent indicator was rs9923231, which alone explained 0.424 of dose variability (indicated by adjusted r2). Adding age increased the prediction of the model to 0.482, then by adding rs61742245 r2 reached 0.507; which suggests that 50.7% of the average warfarin dose in our sample could be explained by genotype at rs9923231 and rs61742245 and age (P<0.05) (table2). Discussion: Indigenous citizens of the United Arab Emirates are considered an admixed population that is under-represented in pharmacogenomics studies. In our group, the comparison of minor allele frequencies (MAF) at the studied SNPs among 90 Emiratis did not yield any consistent pattern or similarity to the earlier studied populations. These findings are not surprising in an admixed population, but it highlights the need for more genomic research in such under-represented group. The intronic variant rs9934438 has been found in near perfect linkage disequilibrium with the promoter variant rs9923231 (LD pairwise r2=1). In our group, around 42.4% of warfarin dose variability could be explained by either of these two variants. This prediction power is more than Caucasians (18%) [7], Iranians (20.3%) [8], African Americans (5%) [9] and close to Omani (45%) patients [10]. The G>T at rs61742245 was found to be associated with the requirement of high doses of warfarin in sporadic reports [5]. It is mainly identified among Ashkenazi Jews and Ethiopians with MAF around 4% and 15%, respectively [11, 12]. rs61742245 was a significant predictor in the regression model in our patients. Our study is the first to examine this variant in Arabs. Moreover, due to its low MAF, few studies have ever evaluated its effect on warfarin dose in other populations. Noteworthy, that five SNPs showed strong LD (rs9923231, rs9934438, rs8050894, rs2359612, rs7294), which was strongest at the first four (r2 > 0.93) and moderate at rs7294 (r2 = 0.7) (figure1). Similar findings were reported from some other populations [4, 7]. When SNPs are in a strong LD, alleles of some of these variants can be predicted from other alleles. Hence, testing all the alleles will give redundant information [13]. Accordingly, in our population rs9923231 and perhaps rs7294 can be chosen from the five previous SNPs, besides the significant variant at rs61742245, in case a limited number of VKORC1 SNPs are to be assessed. Haplotype analysis based on star system did not
give a significant association with dose. In contrast, haplotypes relying on the six variants that have shown significant outcomes in our primary variance analysis gave a significant association with dose. A better haplotype/dose model would be constructed if more variants were included and variants from other genes were tested. Regarding the non-genetic factors, gender and age were examined. While the gender did not show any effect on dose, age was the second most reliable dose predicting variable. It is already established that patients of older age have to be treated with more conservative regimens of warfarin. The higher risk of bleeding, co-morbidities, and concomitant used medications all are among the leading reasons for starting with lower doses in geriatric patients [14]. Conclusion: This is the first report of the explanatory power of VKORC1 genotypes and non-genetic factors (age and gender) on warfarin dose in Emiratis. The common variant rs9923231 along with the rare variant at rs61742245 and age attributed to more than 50% of the variability in warfarin dose. Although VKORC1 genotype and age are well-known predictors of warfarin dose in different populations, a model based on these factors was remarkably a strong predictor of warfarin dose in our population. We highlighted here that under-presented populations exhibit different allele frequencies, haplotype structures, and might have some rare actionable pharmacogenomic variants.

Reference:

Attachment:
Al Mahayri et al. figure and tables:
http://membs.org/membs/uploads/congressSpeaker_files/1527663518Almahayri et al. Figure and tables.docx
Exploring the Active Center of the LSD1/CoREST Complex by Molecular Dynamics Simulation Utilizing Its Co-crystallized Co-factor Tetrahydrofolate as a Probe

Zalloum, Waleed (1), Zalloum, Hiba (2)

(1) Assistant Professor, Department of Pharmacy, American University of Madaba, Jordan.
(2), , The University of Jordan, Jordan.

Abstract:

Waleed A. Zalloum* and Hiba M. Zalloum* Abstract Epigenetic targeting of cancer is a recent effort to manipulate the gene without destroying the genetic material. Lysine-specific demethylase 1 (LSD1) is one of the enzymes associated with the chromatin for post-translational modifications, where it demethylates lysine amino acid in the chromatin H3 tail. Many studies showed that inhibiting LSD1 could potentially be used to treat cancer epigenetically. LSD1 is associated with its corepressor protein CoREST, and it uses tetrahydrofolate as a cofactor to accept CH2 from the demethylation process. In this study, the co-crystallized co-factor tetrahydrofolate was utilized to determine possible binding regions in the active center of the LSD1/CoREST complex. Also, the flexibility of the complex has been investigated by molecular dynamics simulation and subsequent analysis by clustering and principal component analysis. This research supported other studies and showed that LSD1/CoREST complex exists in two main conformational structures: open and closed. Furthermore, this study showed that tetrahydrofolate stably binds to the LSD1/CoREST complex, in its open conformation, at its entrance. It then binds to the core of the complex, inducing the closed conformation. Furthermore, the interactions of tetrahydrofolate to these two binding regions and the corresponding binding mode of tetrahydrofolate were investigated to be used in structure-based drug design. This work has been published in Journal of Chemical Information and Modeling (impact factor 3.76). It is worth mentioning that these models were used to conduct a docking study for organic compounds’ database such as NCI. Then, the best score compounds were tested against different cancer cell lines, such as prostate cancer and neuroblastoma. This approach was successful since we get compounds with IC50 in low micro-molar concentrations.
Cancer, Genetics, Diseases, Immunology, Histopathology

**Vitamin D Receptor Gene Polymorphisms Expression (BsmI & FokI) and the Risk of Breast Cancer: Correlation with the Expression of B Cell Lymphoma-2 (Bcl-2) and Clinicopathological Features of the Disease.**

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**Abstract:**

Breast cancer (BC) represents the most common cancer among women, and its rates are increasing in developing countries, including Egypt. The American Cancer Society estimates that 234,190 Americans will be diagnosed with invasive breast cancer and 10,730 will die of the disease in the United States in 2015. In Egypt, breast cancer is the most common cancer among women, representing 33.5% of newly diagnosed cancer cases. In Alexandria, it accounts for 45.4% of all malignancies in females during the year 2012. We aimed in this study to investigate whether the different specific vitamin D receptor (VDR) gene polymorphism expression are associated with breast cancer risk in Egyptian women. In addition, serum level of 1?,25-dihydroxyvitamin D3 as well as B cell lymphoma-2 (Bcl-2) level were analyzed and correlated to vitamin D receptor gene polymorphism and to different clinicopathological features of the disease. The study was conducted on forty five females; thirty women with different stages of breast cancer, and fifteen normal healthy females were included as a control group. All patients under study were subjected to full history taking and clinical examination. Fresh blood samples were obtained from all subjects, serum separation was done for measurement of Vitamin D and B cell lymphoma-2 (Bcl-2) levels by ELISA technique, and peripheral blood mononuclear cells (PBMCs) were isolated for DNA analyses for VDR gene polymorphism expression using real-time PCR technique. In our study, Egyptian breast cancer patients and healthy women were analyzed for VDR gene Bsml and Fokl polymorphisms. The susceptibility and modifier genes play a major role in developing malignancies in concert with environmental factors. In this respect, VDR polymorphisms in breast cancer pathogenesis have attracted much interest worldwide. The effects of Vitamin D on proliferation and differentiation of several cancer types have been demonstrated. The distribution of two VDR gene SNPs, Fokl and Bsml, has been investigated in breast cancer patients. The present study demonstrated that a significantly increased risk of breast cancer was observed with Bsml Bb genotype. The data suggested that the b allele may contribute in susceptibility to breast cancer, either in heterozygote or homozygote state. When the Ff genotypes were compared with the reference genotype, a significant with increased risk of breast cancer was observed with Fokl FF genotype. In the present work, the results indicated that no significant differences between the mean values of vitamin D and the three categories of Fokl- (FF, Ff, ff). But, data showed high significant differences between mean values of vitamin D at the three categories of Bsml- (Bb, Bb) compared with recessive category (bb). Also, data showed a decreasing pattern in the mean value of vitamin D with advancing stages of Breast Cancer. This result indicates that there is a significant difference between the mean values of vitamin D for the tumor grades. So, we can say that the less concentration of vitamin D, the more advanced grade of the breast cancer. Our results showed that there were significantly higher serum Bcl-2 levels in breast cancer patients before surgery than in normal healthy controls. The results obtained also revealed that the mean value of Bcl-2 for the breast cancer patients was significantly higher than the control group. We almost can say that the Bcl-2 values would increase with advanced stages of breast cancer. Our results showed that there was a significant negative association between Bsml genotype and vitamin D levels. In addition, another significant negative correlation was found between vitamin D levels and CD56+CD16- percentage. Our study support potential effects of VDR polymorphisms vitamin D deficiency and possible differential effects of breast cancer risk. We recommended providing the grounds to justify clinical efforts to maintain and improve a patient’s through vitamin D supplementation or lifestyle modifications, to enhance breast cancer patients’ survival particularly in advanced-stage cancer and older patients. Our study suggests that serum Bcl-2 is good diagnostic and prognosis monitoring biomarker for breast cancer patients. Our results supported the growing evidence for a protective effect of vitamin D on the risk of breast cancer. We recommended future studies on the relationship of VDR polymorphism and breast cancer should take into account the potential modifying effect of family history of breast cancer in first-degree relatives.

**Reference:**

The effect of endometrial injury on HLA-A and E expression in patients with Repeated Implantation Failure.

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Abstract:

Human embryo implantation is mediated by a complex interaction between embryo and endometrium, which is necessary for successful pregnancy. The failure of implantation may be decreased endometrial receptivity, defective embryonic development or both of them. Repeated implantation failure (RIF) is an unknown major barrier of fertility in infertile women. It is assumed that a defective receptive endometrium is the main cause of RIF. Many strategies have been done to improve the implantation rate in RIF patients. Endometrial injury (EI) is one of these procedures which recently received more attention. However, the mechanisms of EI remains controversial. It seems HLA-A and E (human leukocyte antigens (HLA)) have an important role in successful implantation. The aim of this study was to determine the effects of EI in follicular phase on HLA-A and E genes expression in patients suffering from RIF. Based on previous studies, local injury to endometrium in luteal and /or proliferative phases of menstrual cycle has conflicting effect on implantation and clinical outcomes, however, little is known about molecular aspects of injury in patients with Repeated Implantation failure preceding IVF/ICSI. Material and methods: A total of twenty women with repeated implantation failure (RIF) who failed to conceive during two or more IVF/ICSI cycles and embryo transfer (ET) participated in this randomized controlled trial (RCT) study. Pipelle endometrial sampling was done twice: One in the follicular phase and again in the luteal phase in case group (N=10) but it was done once in control group (N=10) just in the luteal phase for genomic evaluation. Results: Total RNA were extracted from endometrial tissues, Then HLA-A and B genes expression were investigated by quantitative real-time PCR. HLA-A and B genes expression were detected in endometrial samples of both groups. Discussion: As a whole, our study provided molecular evidence that patients with repeated implantation failure can benefit from local injury during an ongoing IVF cycle. Keywords: Infertility, HLA-A, HLA-E, Repeated implantation failure
Abstract:

Cancer is considered a leading cause of death worldwide according to World Health Organization. However, the traditional chemotherapy suffers from serious side effects and the developed of resistant tumors. Combined therapy through the combination of multiple treatments with various mechanisms is a promising and interesting strategy to treat cancer, as it can reduce the toxic side effects of the tolerated dose of the chemotherapy and also allow the developed co-delivery system with synergistic therapeutic effects. One of the co-delivery systems that have shown interesting results in cancer therapy is the co-administration of an anticancer drug with small interfering RNA (siRNA). However, there are still various obstacles which prevent the practical use of this approach, as the low loading capacity of the siRNA and the anticancer drug and the instability of the siRNA as a drug. Therefore, there is a huge need to develop a new targeted co-delivery system that can carry the combined therapy with high loading capacity and high efficacy. Herein, we will functionalize the single-walled carbon nanotubes (SWCNTs) for the first time with the anticancer drug, doxorubicin along with a siRNA against an antiapoptotic gene and a targeting agent such as glucose. The developed co-nanodelivery system will provide high efficacy of the combined therapy as the SWCNTs have a large surface area that can be loaded with numerous copies of both drugs. This nano-system will show for the first time the triple functionalization of SWCNTs with the purpose of delivering chemical and biotherapeutic drugs and will be a promising candidate in the field of cancer therapy.

Reference:

Anti-inflammatory property of Varthemia iphionoides extracts in in vitro cell models of prostate cancer and fibroblasts

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Abstract:
Varthemia iphionoides is a medicinal plant grown in Jordan that have various health-promoting activities, such as antibacterial (1, 2) and anticancer (4, 5) activities. However, studies on its anti-inflammatory properties are limited. The present study aimed to explore the anti-inflammatory property of V. iphionoides through measuring the secretion of interleukin-6 in response to a bacterial lipopolysaccharide, a pro-inflammatory stimulus, in in vitro cell models of human MRC-5 and PC3 cells. The results indicated that there was a significant decrease in the levels of lipopolysaccharide-induced interleukin-6 secretion in response to V. iphionoides (125 µg/mL) in both non-cancerous fibroblast MRC-5 and prostate cancerous PC3 cells. However, the observed anti-inflammatory effect of this medicinal plant was in response to the aqueous extract in MRC-5 cells, and in response to methanolic extract in PC3 cells. The results also indicated that the reduction in the levels of interleukin-6 was not due to the cytotoxic effect of V. iphionoides. Further studies are needed to identify the phytochemical compounds responsible for this effect and explore the mechanisms of action by which this medicinal plant control the inflammatory responses. In conclusion, the results of current study considered the first report of the potential protective effect of water and methanolic extracts of V. iphionoides against pro-inflammatory stimuli in fibroblasts and cancer cells of human origin.

Reference:
THE EFFECTS OF ERCC1 EXPRESSION LEVELS AND POLYMORPHISM ON THE CHEMOTHERAPY RESISTANCE OF BREAST CANCER PATIENTS TREATED WITH PLATINUM BASED ADJUVANT CHEMOTHERAPY

AUTHORS: SUHAD KHALID KARIM BAN ABAAS ABDUL MAJEEED MUHAMMAD IBRAHEEM NADER IRAQ / UNIVERSITY OF BAGHDAD.

khalid, suhad (1), Abbas Abdul-Majeed, Ban (2), Ibraheem, Mohammed (3)

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(2) Consultant , Molecular Pathology and Genetics, Al Nahrain University, Iraq.
(3) Head of the Department , Genetic Engineering , Genetic Engineering and Biotechnology Institute / University of Baghdad., Iraq.

Abstract:

Background and objective: The function of the excision repair cross-complementation group 1 gene ERCC1 impacts on the DNA damage response, particularly in anticancer therapy. The objective of this study is to assess the joint effect of ERCC1 mRNA expression and genotype on breast cancer patients' response to platinum-based chemotherapy. Methodology: This is the case control study involving 44 breast cancer patients on chemotherapy and 31 apparently healthy women involved as a control. Genomic DNA and RNA were extracted from patients' blood. ERCC1 and reference gene (?-actin) cDNA fragments were amplified by Real time qPCR and subjected to relative quantification. Genomic DNA was subjected to genotyping by Taqman Real time PCR to study the SNPs. Results: A significantly higher expression levels of ERCC1 was detected in patients than controls. Genotyping analysis revealed that patients carrying the C/T and T/T genotypes of rs 16115 of ERCC1 had higher gene expression whereas the ERCC1 C8092A rs3212986 SNP did not associate with breast cancer risk. Conclusion: The presence of mutant allele of ERCC1 at the rs16115 was associated with an increase mRNA level suggesting its possible effect on resistance to chemotherapy

Reference:


Attachment:
figures and tables:
http://membs.org/membs/uploads/congress_speaker_files/1527707240Figure and table to membs.docx
Genes involved in interaction embryo-endometrial in natural cycle in mice

ajdary, marziyeh (1), Aflatoonian, Reza (2), mehdizadeh, mehdi (3), Amjadi, Fatemehsadat (4), Zandieh, zahra (5)

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Abstract:

Aim and background: Embryonic implantation, the process by which the human embryo orientates towards, attaches to and finally invades the underlying maternal endometrial tissue, requires a receptive endometrium, a functionally normal blastocyst and an adequate cross-communication between them. Many molecules take part in the dialogue between the human blastocyst and the maternal endometrium to achieve implantation. Here, we evaluate genes that involved in Embryo-endometrial interaction and aimed to compare the expression of endometrial receptivity markers, including homeobox gene 10 (HOXA10), leukemia inhibitory factor (LIF), Epidermal growth factor (EGF), leukemia inhibitory factor receptor (LIFR), Mucin 1, cell surface associated (MUC1), Progesterone receptor (PGR), Fibroblast growth factor 2 (FGF2), Heparin-binding EGF-like growth factor (HBEGF), Colony stimulating factor 1 (CSF1), Vascular endothelial growth factor A (VEGFA) as well as genes increasing during the implantation window in mice natural cycles with control group.

Material & Methods: Twelve 8-week-old female NMRI mice were randomly divided into 2 groups, pregnant group which were mated in sterus cycle and control group which were mated to vasectomized male mice. Successfully mated female animals were identified with vaginal plugs designated gestation day 1. At day 4.5, pregnant donor mice were euthanized, and uterus samples were collected for quantitative polymerase chain reaction analyses. Results: Messenger RNAs (mRNA) expressions LIF, HBEGF, MUC1, PGR, HOXA10 were high significant (p<0.05) in the pregnant group compared to the control group. Conclusion: We find an association between the endometrial and embryo, we noticed an increase in genes involved implantation with presence embryo in endometer, which may be a factor for determined implantation.
In Silico Analysis of Single nucleotide polymorphism in Human DCDC2 gene

ShaikhEldeen Omer, Rufaida Omer (1), ShaikhEldeen Omer, Rufaida Omer (2)

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(2) Master Student in bioinformatics, Bioinformatics, membs _ Sudanese biologists organization, Sudan.

Abstract:

Dobulecortin domain 2 gene (DCDC2) encodes for the doublecortin protein is located in chromosome 6p22.3. The doublecortin domain has been demonstrated to bind tubulin and enhance microtubule polymerization. Variants of the DCDC2 gene may affect the protein conformation and structure which might lead to learning disability (Dyslexia disease). The aim of this study was to analyze the genetic variation that can alter the expression and the function in DCDC2 gene using computational tools. This study focused on the coding region. The total number of SNPs was obtained from dbSNP database. A total of 21 SNPs were found to be damaging by both SIFT and PolyPhen. When using I-Mutant 3.0, 19 SNPs showed decreased protein stability while only 2 showed increase in protein stability. This gene was found to co-expressed with 16 other genes and has a physical interaction with 1 gene using GeneMANIA software. A structural and functional analysis of ns SNPs was also studied by Project HOPE and Mupro softwares. Based on this work, four new ns SNPs are predicted to have pathological effect, thus we proposed that the most deleterious ns SNPs with an SNP ids ((rs375996594) was the most important one. The others (rs372751993), -(rs368811196)and- (rs141060456) may also play an important role in investigation of dyslexia disease among patients.

Reference:

EVALUATING THE DEGREE OF OXIDATIVE DNA DAMAGE & APOPTOSIS IN HUMAN LYMPHOCYTES CULTURED IN THE PRESENCE OF BETA-CAROTENE USING COMET ASSAY, AND FAS L (CD95)

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(1) professor , chemistry & biochemistry, ISEM / and College of Medicine / Al-Nahrain University, Iraq.

Abstract:

Background: Reactive oxygen species can cause various damage to different parts of the body, including the blood. It can damage the proteins, lipids as well as the DNA components of the blood. Oxidative DNA damage can be measured in lymphocytes by various techniques which is a useful way to assess the overall degree of oxidative stress. This study measures the levels of DNA damage and assess the proportion of the DNA cellular repair in human lymphocytes cultured in vitro conditions, the impact of the presence of beta-carotene using comet assay (single cell gel electrophoresis), and to evaluate the apoptosis in these cells if it may occur. Subjects and Methods: The study included 50 individuals aged between 20-50 years, during the period from October 2014 to November 2015. All participants were healthy, non-smokers with no family history of any disease. Also, they were not taking any type of vitamins or dietary supplements. Ten milliliters of total blood sample were collected in heparinized containers. Random blood samples of ten participants (5 males, 5 females) were used to study the effects of different concentrations of beta-carotene (100 and 10000) µg/ml on cultured lymphocytes by trazoleom assay. Samples from the remaining 40 participants were used to assess the levels of DNA damage in cultured lymphocytes using single cell gel electrophoresis in presence of the two different concentrations of beta-carotene (100 and 10000 µg/ml) and also to measure Fas L (CD95). Results: treating the lymphocytic cells with hydrogen peroxide caused a considerable damage to the DNA, however, lymphocytic cells treated with beta-carotene (at concentrations of 100 and 10000 Ug/ml) showed less significant DNA damage. This was also associated with a significant change in the average tail lengths (in comet assay), indicating the positive effects of beta-carotene on the lymphocytes. Fas L (DC95) was not detected among the healthy lymphocytes, which was not associated with lymphocytic stimulation in response to beta-carotene. Conclusions: This study proved the beneficial antioxidant capacity of beta-carotene in reducing the degree of the oxidative stress as manifested by the high levels of oxidative DNA damage measured via comet assay. A high concentration (10000ug/ml) of beta-carotene proved to be highly Beneficial to the lymphocytes compared to a lower concentration.

Reference:

**Abstract:**

Background Duvenhage rabies virus belongs to Lyssavirus genus family Rhabdoviridae causing fatal infection with no effective treatment available and no licensed DNA or peptide vaccine up to date. The aim of present study was to predict peptide vaccine for Duvenhage rabies virus. Methods and Materials The sequences of Duvenhage virus was optioned from NCBI, and then it was subjected to many B cell and T cell tests from IEDB to realize the most promising peptides that could act as driven-peptide vaccine. Population coverage analysis was performed for selected peptides using IEDB and finally homology modeling and molecular docking studies were done to visualize the interaction with MHC1 molecules. Result and Conclusion Among the tested peptides for T cell-test, this study projected an interesting epitope of T cell (YFLIGVSAV) that exhibit all-consuming of binding affinity as strong indicator to MHC-One and MHC-two alleles together, besides the binding to eighteen alleles through the population coverage 99.36% in the world. These results were further supported by molecular docking studies that show excellent interaction with MHC homo spins molecule with the lowest binding energy among tested peptides. Only three B cell epitopes (AHYK, YTIPDKL and SLHNPYPDSH) were found to overlap all performed B cell tests by being linear, on the surface of glycoprotein G and being antigenic.

**Reference:**

Identification of Two Novel Homozygous 5’ Donor Splice - site IVS1+1G?T and Missense G>A (Asp 413 Asn) Mutations in the Factor X Gene in Unrelated Palestinian Families. Riham Smoom, Imad Abushkeidem and Hisham Darwish Department of Biochemistry, Faculty of Medicine and the Molecular Genetics Lab, Al-Quds University, Abu Dis, Jerusalem, Palestine

Smoom, Riham (1), Darwish, Hisham (2)

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(2) professor, Molecular genetics lab, Al- Quds University, Palestine.

Abstract:

Abstract Factor X deficiency is a rare autosomal disease with an estimated prevalence 1: 1,000,000. It is characterized by a reduction in factor X, an essential component of the prothrombinase complex responsible for converting prothrombin to thrombin. The aim of the study was to identify the molecular defects in the factor X gene in Palestinian factor X deficient patients. Nine unrelated Palestinian patients were identified by thrombin time [PT], activated partial thromboplastin time [APTT] and plasma factor X levels. All exons including exon/ intron border and promoter regions were PCR amplified, purified, sequenced and compared to the normal factor X gene. A novel splicing junction mutation IVS1+1G?T was identified in two patients resulting in a major distortion of the protein structure and function. A second novel missense mutation Asp413Asn G>T that apparently distorts the protein structure and affects its catalytic activity was identified in the family of one patient. Six patients proved to be homozygous of the previously identified c358 delG deletion mutation leading to a severely truncated protein that seems specific to our population. A putatively mild heterozygous mutation Ser105thr G>C was detected in two patients who suffer from the severe c358 delG deletion mutation.

Attachment:

Molecular study Usher syndrome in the Algerian population

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Abstract:
Background: Usher syndrome is an autosomal recessive disease that associates sensorineural hearing loss, retinitis pigmentosa and, in some cases, vestibular dysfunction. It is clinically and genetically heterogeneous. To date, 10 genes have been associated with the disease. Method: In a cohort of 12 young patients clinically diagnosed as Usher syndrome, we searched for mutations in known Usher genes, using targeted exome of Usher syndrome genes. Results: Mutations were found in all patients, the USH1 is a priority with 10 mutations. Conclusion: This study underlies the importance of early molecular diagnosis of Usher syndrome in Algerian population. Thus, children can benefit from a cochlear implant.
Explore the expression levels of matrix metalloproteinases inhibitor TIMP1, TIMP2 and MMP2, MMP9 in colon cancer

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Abstract:

Background Colon cancer (or CRC) is the third most common cancer worldwide, responsible for more than half million deaths every year. Metalloproteinases (MMP2 and MMP9) participate in tumor invasion and metastasis due to their ability to degrade extracellular matrix (ECM). Methods: The expression levels of MMP2 and MMP9 as well as TIMP1 and TIMP2 in Iraqi patients with colon cancer were determined by immunohistochemistry in 42 patients and in 18 benign tumors. Results: Immunohistochemistry revealed that the protein expression levels of MMP2 and MMP9 as well as TIMP1 and TIMP2 were increased in 83%, 72%, 64% and 57% in patients with colon cancer tissues as compared to 19%, 28%, 23% and 16% in benign tumor tissues. Conclusion: MMP2 and MMP9 as well as TIMP1 and TIMP2 expression levels in Iraqi patients with colon cancer could be used as potential markers for prediction of tumor behavior, progression and prognosis. Key words: Colon cancer, MMP2, MMP9, TIMP1 and TIMP2 , IHC
Changes in Tumor necrosis factor (TNF-?), its receptors (TNF-? R1 and TNF-? R2) and nuclear factor kappa B (NF-?B) in Brain tissues of Collagen-Induced Arthritis (CIA)

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(3) professor of pathology, pathology department, faculty of medicine, menofia university, Egypt.

Abstract:
Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that leads to progressive joint deformity and disability, and increase the morbidity and mortality due to cardiovascular and cerebrovascular diseases. The experimental model of Collagen-induced arthritis (CIA) has similar manifestations with those of RA. Cytokines, are specialized molecules of the immune system, are now being investigated also for their synaptic and inflammatory action on the central nervous system (CNS), and contribute to two main types of action: modulation of neuronal excitability and local inflammatory responses. Materials and Methods: Tumor necrosis factor (TNF-a), its receptors (TNF-R1 and TNF-R2) and nuclear factor-?B (NF-?B) was evaluated in brain tissues of collagen induced arthritis (CIA) rat model under methotrexate (MTX) treatment. Rats were divided into: normal rats and CIA model before and after MTX treatment. Whole marks of arthritis were examined by ultrasonography. Pathological changes (degree of: red neuron, neuronal degradation, gliosis, microglia proliferation, congestion, edema, inflammatory cell infiltrate, demilination, and apoptosis) were examined. TNF-?, TNF-? R1, TNF-? R2 and NF-?B were quantified by enzyme-linked immunosorbent assay (ELISA) in brain tissue homogenate. Results: Levels of TNF-a, TNF-R1 and NF-?B were increased in CIA, with a remarkable reduction after MTX treatment. While TNF-R2 was decreased in CIA, it was significantly increased by MTX treatment. Most of tested neuronal changes was absent in control groups and appeared with significant frequency in CIA and MTX groups. Conclusion: To our knowledge, this study is the first one that study cytokines in brain tissues of CIA rat model. The present data give some light about the participation of inflammatory mediators in RA not only in bone but also in brain tissues. The observed pathological changes appeared in CIA foes not disappear by MTX treatment. It is mandatory to put the neurological manifestation as one of the weighted factor in any drug choice for RA treatment. The emerging picture suggests that cytokine expression in the brain serves a fundamental need for integration of molecular and cellular activities. Thus, we need to further investigate how and why cytokine signals propagate through the brain, especially in RA patients.
EFFECT OF ADIANTUM CAPILLUS VENERIS L. AQUEOUS EXTRACT ON HYPERCHOLESTEROLEMIA

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(5) Senior Researcher, Center of Bioanalysis, University of Bucharest, Romania.
(6) laboratory technician, Faculty of pharmacy, University of Jordan, Jordan.

Abstract:

Adiantum capillus veneris L. (Adiantaceae) has been reported as herbal medicine for diverse disorders in Jordan. However, its assumed benefits and mechanism of action remain obscure. Thus the current study aimed to identify its phenolic constituents of by LC-MS and to evaluate the chronic effect of its water extract on 10-weeks high cholesterol diet (HCD)-fed rats and atherogenic index. Rats were divided into four groups of 6 rats each. Group 1 was kept on stand diet; the remaining three groups were fed high-cholesterol for ten weeks. On week 7 onwards; Group 3 was given atorvastatin of concentration 10 mg/Kg/b.wt, while group 4 was given daily aqueous extract of A. capillus veneris of 500 mg/Kg/b.wt. HPLC-MS analyses revealed the presence of ellagic acid, rutin, quercetin-3-O-glucoside, ferulic acid, gallic acid, caffeic acid, epicatechine and quercetin. 10-week administration of A. capillus veneris extract in HCD-fed rats decreased highly significantly the total cholesterol (TC), LDL and VLDL serum levels. VLDL serum levels in both intervention groups were substantially (p<0.001) and comparably decreased. Neither treatment could affect HDL serum levels. Besides, atherogenic index TC/HDL parameter was normalized in A. capillus veneris-treated rats. A. capillus veneris can be considered as potential candidate for management of hypercholesterolemia and its atherosclerotic complications.
Molecular Diagnostic Testing of Solid Tumors from International Guide Lines to Local Practice

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(6) Senior Clinical Scientist, Diagnostic Genomic Division, Hamad Medical Corporation, Qatar.

Abstract:

Introduction: Molecular genetics biomarkers are widely used as diagnostic, prognostic and predictive tool for many cancer conditions. For several cancers, established guide lines are involve the molecular biomarkers which improve the cancer patient’s management and also increase the clinical impact of response to treatment. Aim: To set up molecular testing work flow according to standard guide lines to improve local services for cancer patients. To introduce molecular testing for detection of somatic variants in DNA/RNA extracted from formalin fixed paraffin embedded (FFPE) tissue collected from cancer patients. Method: Different technologies were used to establish cancer testing services according to standards set by the College of American Pathologist (CAP). These techniques include Real Time – PCR and BIOCARTIS, Sanger sequencing and next generation sequencing (NGS). A Testing strategy was developed to improve the turnaround time (TAT) to overcome the limitation of each technique and to provide comprehensive testing services. Results: Testing for several genes that contain molecular biomarkers were successfully implemented in Diagnostic Genomic Division (DGD) at Hamad Medical Corporation (HMC). These genes are KRAS, BRAF, NRAS, EGFR, cKIT, PDGFRA and IDH1 & IDH2. Based on our validation study, limit of detection for each used method was as follow: Real - Time PCR and BIOCARTIS with up to 1%, Sanger sequencing 20% and NGS 5% .Patients with several cancer types, including colon, lung, brain, melanoma and GIST were beneficiary of this service. Since November 2016, approximately 300 cases were tested; the highest referrals were for lung and colon cancer. The TAT reduced from 1 moth to 2 days for some tests. Conclusion: Providing molecular testing for cancer patients have improved the clinical outcome by providing results in short TAT especially for patients who needed targeted therapy.

Reference:

Exome Reanalysis Identifies A Recessive Truncating Variant In Thrombospondin-1 Domain Containing Protein 1 Gene THSD1 As The Underlying Cause Of Non-Immune Hydrops Fetalis, Congenital Cardiac Defects, And Haemangiomas In 4 Patients From A Consanguineous Family

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(2) Paediatric physician, Genetics Clinic / Paediatrics department, Tawam Hospital, United Arab Emirates.
(3) Professor of Molecular and Genetic Medicine, Department of Pathology, College of Medicine d Health Sciences, UAE University, United Arab Emirates.
(4) Professor, Pediatrics, UAEU, United Arab Emirates.
(5) Senior research specialist, Pathology and Genetics, College of medicine and health sciences, UAEU, United Arab Emirates.

Abstract:

Non-immune hydrops fetalis (NIHF) is the abnormal accumulation of serous fluid in more than two fetal or neonatal interstitial spaces due to non-immune causes. It is a serious condition that requires extensive medical care as it indicates severe fetal compromise. We clinically evaluated four patients from two branches of a highly consanguineous family from the UAE with NIHF using whole exome sequencing and in silico analysis. Fetal onset pleural and peritoneal effusions were detected in all 4 patients and were born with moderate to severe hydrops fetalis that resolved with age. Follow up showed relatively normal growth and development apart from mild ascites and haemangiomas in all affected children, recurrent hydrocele in all affected males, intestinal malabsorption in 2 patients, dysmorphic features in 2 patients, and congenital cardiac defects in 3 out of 4 patients. Exome sequencing in 2015 was unfruitful. However, raw data reanalysis in 2017 identified a homozygous 8 nucleotide deletion in THSD1 gene (NM_199263:c.1163_1170delGGCCAGCC, p.Arg388Glnfs*66) as the underlying cause of this phenotype in the affected children. The novel variant co-segregates with the described phenotype in an autosomal recessive mode of inheritance and is predicted to be pathogenic as it leads to a truncated protein that lost important structural and functional domains. Thrombospondin-1 domain containing protein 1 gene THSD1 has been recently associated with an autosomal recessive form of NIHF and embryonic lethality (Shamseldeen et al., 2015). Interestingly, THSD1 pathogenic variants were also found associated with intracranial aneurysms. In a recent article, Santiago-Sim and colleagues have identified 6 sporadic and 2 familial heterozygous variants in THSD1 in 18 middle-aged to elderly patients (aged 35-71 years) diagnosed with intracranial aneurysms (Santiago-Sim et al., 2016). Rui and colleagues have recently reported that THSD-1 protein interacts with nascent focal adhesion proteins in extracellular matrix and enhances the formation of a multimeric molecule of the scaffold proteins talin, vinculin and focal adhesion kinase that enhance nascent adhesions formation (Rui et al., 2017). It appears that these interactions are affected by pathogenic missense variants in intracellular domains as shown in the co-immunoprecipitation experiments by Rui and co-workers. However, due to the truncation effect of the variant p.Arg450Ter they were not able to co-immunoprecipitate the mutant protein with any of the scaffold proteins (Rui et al., 2017). We predict that the truncated protein in our patients will similarly fail to interact with talin and other scaffold proteins affecting the integrin-extracellular matrix interactions in blood vessels. Therefore, given the available evidence on the THSD1 function, one can speculate that the underlying pathophysiology of fetal hydrops and polyhydramnios in our patients is increased vascular permeability due to the disruption of the physical structure of the pores in the microvascular membrane rendering it leakier to fluid and macromolecules to interstitial spaces. This microvascular weakness may also explain the higher tendency to aneurysms. In conclusion, in this poster we show that THSD1-associated NIHF segregate in an autosomal recessive mode of inheritance. We describe in detail the variable clinical picture of NIHF accompanying symptoms, thus expand the phenotype and identify the infantile hemangiomas as a common feature among affected children. It is of value to consider clinical exome/genome analysis for unexplained NIHF patients as it expands the genetic, phenotypic and pathologic spectra of this phenomenon.

Reference:

Ca2+ tunneling as a selective intracellular signaling pathway.

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(1) Assistant Professor of Research in Physiology and Biophysics, Research Dept., WCM-Q, Qatar.
(2) Research specialist, Research Dept., WCM-Q, Qatar.
(3) Professor and Associate Dean of Research, Physiology and Biophysics, Weill Cornell Medicine, United States.

Abstract:

Intracellular calcium is the most ubiquitous second messenger and is central to key events such as fertilization and cell death. Cells have two main sources connected to the cytosol through ion channels to create intracellular Ca2+ signals: the endoplasmic reticulum (ER) and the extracellular space. Because of the multitude of Ca2+ targets in a cell, the signals have to be very precisely encoded in time and space and this is regulated by physical barriers, pumps and Ca2+ buffers. The relative localization of the Ca2+ source and of the Ca2+ targets are therefore of key importance. In the case of Store Operated Ca2+-entry (SOCE), effectors have to be located in the immediate vicinity of the Ca2+ source as SOCE cannot activate efficiently targets away from the plasma membrane (PM). One way to deliver Ca2+ from SOCE to distant targets is to use the tunneling process where the ER cisterns are used as a pipeline taking Ca2+ from the SOCE microdomain and releasing it through IP3Rs to distant effectors. Here we used HeLa cells to study the effect on specific intracellular targets of various Ca2+ mobilizing pathways: SOCE, release from the ER and tunneling. In HeLa cells mitochondria localize away from the PM and constitute therefore a candidate of choice for tunneling. When Ca2+ is released from the stores following opening of IP3Rs, mitochondrial Ca2+ levels follow the rise in cytosolic Ca2+. In contrast, the mitochondria did not respond to Ca2+ influx through SOCE. Surprisingly, during tunneling, the large cytoplasmic rise observed was not associated with a significant change in mitochondrial Ca2+. Detailed analysis of the relative localization of the mitochondria and of the ER during store depletion failed to explain the differences in mitochondrial signaling. The kinetics of the cytosolic Ca2+ increase induced by tunneling was however slower than ER release, presumably due to the slow pumping in the ER by the SERCA as compared to Ca2+ release through the IP3Rs. Conversely, when the target was a Ca2+-activated potassium channel, or the PM itself, tunneling was extremely effective in delivering Ca2+ to these sites, whereas SOCE itself behaved as a poor activator. In contrast, when NFAT translocation was used as a reference for Ca2+ signaling, SOCE was extremely potent while Ca2+ release was ineffective and tunneling only induced a slight enhancement of the SOCE effect. We conclude that Ca2+ entering through SOCE to be release by IP3Rs is preferentially released close to the source (i.e the PM) and generates a weaker rise deep in the cell unable to reach the required conditions to trigger Ca2+ increase in the mitochondria. Recent findings suggest that active IP3Rs indeed located close to the PM surrounding SOCE clusters(1). It is therefore clear the spatio-temporal properties of the Ca2+ increase induced by tunneling contribute to activate selective intracellular Ca2+ targets.

Reference:

A Novel Disease-Causing AMPD2 Variant In A Patient With Pontocerebellar Hypoplasia 9 And Evidence On The Presence Of Potential Pathogenic Variants In Non-Middle Eastern Populations

Abdelrahman, Hanadi (1), Al Shamsi, Aisha (2), Ali, Bassam (3), AlGazali, Lihadh (4)

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(2) Paediatric physician, Genetics Clinic / Paediatrics department, Tawam Hospital, United Arab Emirates.
(3) Professor of Molecular and Genetic Medicine, Department of Pathology, College of Medicine d Health Sciences, UAE University, United Arab Emirates.
(4) Professor, Pediatrics, UAEU, United Arab Emirates.

Abstract:
Pontocerebellar hypoplasia type 9 (PCH-9) is an autosomal recessive neurodegenerative disorder caused by loss of function variants in AMPD2 gene. We clinically evaluated an Emirati patient presented with severe developmental and growth delays. We performed exome sequencing, Sanger sequencing and segregation analysis followed by in silico and in vitro analysis to elucidate the pathogenicity of the variant, we also ran a population ancestry analysis of likely pathogenic variants on ExAC database. We identified the novel mutation (c.1633G>A) in AMPD2 gene. This variant is predicted to be pathogenic using several in silico tools, and resulted in a decrease in the enzyme function in the patient’s polymorphonuclear cells (PMNCs) by 82% (95% CI: 73.3-91.7%, p=0.029) compared to control. This data establishes that the affected child is affected by PCH-9. In addition, population analysis of AMPD2 variants confirmed the presence of potentially disease causing mutations in non-Middle-Eastern populations. Pontocerebellar hypoplasia type 9 (PCH-9) is a recently described, very rare, autosomal recessive neurodegenerative disorder [1]. Affected infants present early with severe developmental delay, spasticity, with MRI picture of thin corpus callosum, atrophied pons and cerebellum. It is caused by loss of function mutations in the AMPD2 gene encoding for the adenosine monophosphate deaminase 2 enzyme. The enzyme catalyses a critical step in the de novo biosynthesis of purines and its deficiency in the developing neurons was found to severely affect neuronal differentiation and cell viability. Only 9 mutations in 13 patients have been reported with PCH-type 9, so far [1,2,3]. Here we describe the clinical presentation and confirm the pathogenicity of a novel missense variant (c.1633G>A, p.Gly545Arg) in AMPD2 detected by exome sequencing in a 3 years old Emirati child with corpus callosum agenesis, brainstem and cerebellar atrophy, and severe developmental and growth delay. The affected child in this report is the third of 4 children to a consanguineous Emirati couple (Pedigree, figure 1). At the time of examination the patient was 3 years old. The patient has severe growth and developmental delay, spasticity, convergent squint, hypoplastic optic discs, seizure disorder, primary microcephaly. MRI at day 2 of age showed agenesis of corpus callosum and atrophied pons and cerebellum (Figure A).

Microarray showed large areas of absence of heterozygosity in chromosome 1 and 22. Whole exome sequencing analysis revealed the novel homozygous variant NM_001257360.1(AMPD2_v001):c.1633G>A, NP_001244289.1 (AMPD2_i001): p. Gly545Arg. In silico prediction reported this mutation as disease causing in MutationTaster. It is also predicted deleterious (score -7.82) in PROVEAN prediction, damaging (score 0.00) in SIFT prediction, probably damaging (score 1.0 – sensitivity 0.0, specificity 1.00) in both HumDiv and HumVar of POLYPHEN2 prediction, and has high functional impact on mutation assessor (FI score 3.88). The position G545 is highly conserved among species (MSA height is 138) The enzyme kinetics showed decreased activity of AMPD2 in the patient’s sample compared to his mother’s. The end point absorbance at 340nm was around 40% less in the patient sample compared to control (95% CI was 29-60%, p= 0.006). The enzyme activity calibrated by the NADH2 absorbance and cell count was mean±SD= 0.002±0.0008 nmol/hr/106 cells in the patient sample, significantly lower than control (mean±SD= 0.01±0.0003 nmol/hr/106 cells). A significant decrease by 0.009 nmol/hr/106 cells (~82%), 95% CI is 0.008-0.01nmol/hr/106 cells (73.3-91.7%) - p =0.029 (figure 2) We found that of all the 825 variants detected in AMPD2 gene, 287 variants of them were missense and INDEL variants. All the 287 variants were analysed using different prediction tools to divide the variants in the following categories: 1. Disease causing 2. Likely pathogenic 3. Alternative splicing 4. Benign/tolerated (likely polymorphism). We extracted the allele and population frequencies for all the likely pathogenic and disease causing variants. Of all 825 variants 142 likely pathogenic variants were distributed over the population groups African, European (Finnish and non-Finnish), Hispanic and Asian (East and South). The results are displayed in figure 3. AMP deaminase catalyzes a critical step in purine de novo biosynthesis. It is also necessary for guanine nucleotide biosynthesis and protein synthesis. Its expression is crucial during neurogenesis and neuronal differentiation [1]. Pontocerebellar hypoplasia type 9, is a severe neurodegenerative disorder with a prenatal onset. All reported patients are from Middle-Eastern origins. Evaluation of genomic public databases give insight on the distribution of genetic variations over different populations, which led us to rule out the confinement of this disease to a specific population. The higher frequencies of this disorder as other rare recessive disorders are explained by the high consanguinity rates in the region. Using WES, segregation analysis, and functional analysis, we identified a novel pathogenic mutation in AMPD2, causing pontocerebellar hypoplasia type 9. This mutation was found to significantly decrease the enzyme activity by 82% in the patient sample compared to his mother’s. Therefore we confirmed the pathogenicity of the mutation and the diagnosis of
pontocerebellar hypoplasia -9 in the proband, and document the first case in the Emirati population. In addition, population ancestry analysis of AMPD2 variants ruled out the possibility that PCH-9 is confined to Middle-Eastern populations.

Reference:

Attachment:
Supplementary figures:
Association of Osteopontin and IL-10 Genes Polymorphism and Risk of Hepatitis C Virus-related Hepatocellular Carcinoma in Egyptian Population

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Abstract:
Hepatitis C virus (HCV) is considered as a major global public health problem. The genetic background may be a crucial etiologic factor in HCV infection and its complications. Osteopontin (OPN) is an inflammatory cytokine belonging to Th1 cytokine family and plays important roles in antiviral host defenses and found to be associated with inflammations and malignancies. Interleukin-10 (IL-10) is a Th2 cytokine that can repress proinflammatory responses and limit unnecessary tissue disruptions caused by inflammation. Mutations in both OPN and IL-10 genes may lead to altered cytokine production and/or activity and thus affects HCV infection outcome. The aim of this study was to investigate the potential associations of single nucleotide polymorphism (SNP) at OPN (-9138A/C) and IL-10 (-1082 G/A) with HCV disease progression and/or HCC in Egyptians. OPN (-9138A/C) and IL-10 (-1082G/A) genotypes were determined in 118 patients with HCV infection and 100 healthy controls, using two different polymerase chain reaction techniques (PCR/RFLP) for OPN (-9138A/C) and (SSP) for IL-10 (-1082G/A). HCV patients were classified into 58 cirrhotic and 60 HCC patients. While OPN -9138CC genotype is strongly correlated with HCV infection, both AC and AA genotypes were correlated with HCC progression. On the hand, there were no significant differences in the genotype and allele frequencies of IL-10 gene polymorphism either between patients and controls or between the two patient groups. Our data stressed the importance of OPN (-9138A/C) SNP in HCV progression. Although, further studies with larger sample size should be conducted to validate these results in the Egyptian population.
Expanding the clinical spectrum of Schindler disease: perinatal presentation

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Abstract:
Background. Schindler disease (MIM 609241) is an ultra-rare autosomal recessive lysosomal storage disorder caused by deficient alpha-N-acetylgalactosaminidase (α-NAGA) residual enzymatic activity. Loss of α-NAGA enzymatic activity leads to the accumulation of glycolipids and glycopeptides in several tissues and urine resulting mainly in neurological symptoms. It is clinically a very heterogeneous disorder with wide range of overlapping symptoms. It is classified into three main types; type I is an infantile-onset neuroaxonal dystrophy, type II (Kanzaki disease) is a mild adult form of the disease characterized with angiokeratoma corporis diffusum and mild intellectual impairment, and type III which is an intermediate form of the disease presented with mild to moderate neurologic manifestations. To date, only 9 mutations have been reported in the NAGA gene of which 7 are missense Schindler disease causing. Schindler disease clinical and molecular picture is not well understood yet and there are a lot of confusion in understanding the genotype-phenotype correlation in patients. Aim. Confirm pathogenicity of a novel missense mutation of unknown significance in the NAGA gene of a three years old child with clinical presentation of Schindler disease and widen the clinical spectrum of the disease. Methodology. In this study, a three years old Emirati male born to consanguineous parents was presented at Twam hospital for clinical diagnosis of Schindler like symptoms. A DNA sample was sent for whole exome analysis followed by Sanger Sequencing for segregation and confirmation of the detected mutations. Skin biopsy and blood samples were collected for NAGA activity measurement via a fluorescence based assay using 4-MU tagged substrate. Results. The child was presented at birth with microcephaly, bilateral dense congenital cataract, talipes equinovarus, dysmorphic features (narrow forehead, microphthalmia, thick and slightly depressed nasal bridge, and relatively big ears). In the neonatal period he had anemia and thrombocytopenia, which is improved subsequently. He has global developmental delay, failure to thrive. Fetal ultrasound revealed increased nuchal thickness, intraruterine growth retardation and normal amniotic fluid. Brain MRI showed microcephaly, cerebral atrophy, reduced sulcation of the cerebral hemisphere, especially frontal and temporal lobes. Lissencephaly with hypoplastic corpus callosum. Cerebral white matter bulk is reduced with delayed myelination. Dysplastic basal ganglia and calcification of periventricular parenchyma around the right lateral ventricle. The intracranial optic nerves and chiasm appear very slender. Whole exome sequencing revealed a novel homozygous c.838C>A variant of unknown clinical significance in the NAGA gene which is predicted to result in a p.L280I substitution at the protein level. The mutated leucine at this position is highly conserved among multiple eukaryotic species indicating its biological importance in NAGA function. c.838C>A was predicted to be deleterious and disease causing via different in silico mutation prediction tools. Pathogenicity of the underlying missense mutation was confirmed with the low detected NAGA activity in patient fibroblast and white blood cells with 13.3% and 7.8% of normal control residual enzymatic activity, respectively. Conclusion. The study has reported the first Emirati case with Schindler disease presented with a novel homozygous c.838C>A missense mutation. The clinical and molecular delineation of the mentioned mutation broaden our understanding of Schindler disease.

Reference:
**National Neonatal Molecular Screening for Sickle Cell Anemia in Qatar**

Alzeyara, Aisha (1), Al Rumaihi, Fatema (2), Abualainin, Wafa (3), Abbaszadeh, Fatemeh (4), Badii, Ramin (5), Saif, Atiya (6), Alavi, Ali (7), Al Mulla, Naima (8), Al-Nabet, Ajaeb (9), Nawaz, Zafar (10)

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(10) Senior Clinical Scientist, Diagnostic Genomic Division, Hamad Medical Corporation, Qatar.

**Abstract:**

Introduction: The diagnostic Genomic Division (DGD) at Hamad Medical Corporation (HMC) established a national newborn screening program for sickle cell anemia in 2012. Method: A combination of techniques was used to test all newborn in the State of Qatar in collaboration with Heidelberg University Hospital in Germany. These techniques included (A) DNA extraction from blood spot collected on Guthrie cards. (B) Genotyping of sickle cell by Real-Time PCR. (C) A confirmatory test arranged for heterozygous cases by RFLP and (D) A confirmatory test for homozygous cases by Sanger sequencing that required fresh blood from the patient. All detected cases were referred to the HMC Pediatric Clinic for follow up and genetic counseling. Results: Approximately 150,000 cases were screened in the past 6 years, of these cases, 1361 cases (1%) were diagnosed as sickle cell carriers (HbAS) and 20 cases (0.014%) were diagnosed as affected (HbSS). Detailed findings, Qatari carriers: 40% (of all carriers), Qatari affected: 4% (of all affected), African carriers: 29%, African affected: 60%, Middle Eastern carriers: 27% and Middle Eastern affected: 24%. Also two complex cases were diagnosed with Sickle- Beta thalassemia and another four with sickle disease associated with HbSC. Conclusion: This screening program has been established for identifying affected cases at an early stage to avoid complications and to reduce unnecessary burden on health care system in Qatar. This experience has also helped us to set up additional screening programs such as neonatal screening for severe combined immunodeficiency (SCID), to help patients for treatment and also improvement of their life style.

**Reference:**

Bender MA. Sickle Cell Disease. GeneReviews. Update of August 17, 2017
T lymphocytes facilitate brain metastasis of breast cancer by inducing Guanylate-Binding Protein 1 expression.

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Abstract:
Pedrosa R1, Sieuwerts A2, Berrevoets C3, Zeneyedpour L4, Priego N5, Valiente M5, Luider TM4, Debets R3, Martens J2, Kros JM1,6, Mustafa DA1,6 1 Department of Pathology, 2 Medical Oncology, 3 Medical Oncology, Laboratory of Tumor Immunology, 4 Neurology, Laboratory of Proteomics, 6 Brain Tumor center, at Erasmus University Medical Center, Rotterdam, The Netherlands. 5 Brain Metastasis Group, Spanish National Cancer Research Center, Madrid, Spain. The discovery of genes and molecular pathways involved in the formation of brain metastasis would direct the development of therapeutic strategies to prevent this deadly complication of cancer. By comparing gene expression profiles of Estrogen Receptor negative (ER-) primary breast tumors between patients who developed metastasis to brain and to organs other than brain, we found that T lymphocytes promote the formation of brain metastases. To functionally test the ability of T cells to promote brain metastasis, we used an in vitro blood-brain barrier (BBB) model. By co-culturing T lymphocytes with breast cancer cells, we confirmed that T cells increase the ability of breast cancer cells to cross the BBB. Proteomics analysis of the tumor cells revealed Guanlyate-Binding Protein 1 (GBP1) as a key T lymphocyte-induced protein that enables breast cancer cells to cross the BBB. The GBP1 gene appeared to be up-regulated in breast cancer of patients who developed brain metastasis. Silencing of GBP1 reduced the ability of breast cancer cells to cross the in vitro BBB model. In addition, the findings were confirmed in vivo in an immunocompetent syngeneic mouse model. Co-culturing of ErbB2 tumor cells with activated T cells induced a significant increase in Gbp1 expression by the cancer cells. Intracardial inoculation of the co-cultured tumor cells resulted in preferential seeding to brain. Moreover, intracerebral outgrowth of the tumor cells was demonstrated. The findings point to a role of T cells in the formation of brain metastases in ER- breast cancers, and provide potential targets for intervention to prevent the development of cerebral metastases.

Attachment:
**Abstract:**

Katanin p80 WD40-containing subunit B1 is a protein that in humans is encoded by the KATNB1 gene. Mutation in KATNB1 have been associated with a condition characterized by severe microcephaly, developmental delay, nonspecific facial dysmorphism and variable abnormalities on brain imaging (eg. lissencephaly, pachygyria, and hypoplasia of the corpus callosum). In this study we report a consanguineous Saudi family with a novel homozygous missense mutation in KATNB1 gene cause microcephaly, facial dysmorphism, brain abnormalities. Whole exome sequencing was performed for the affected members of the family to study the novel mutation. Whole exome sequencing data analysis, confirmed by subsequent Sanger sequencing validation, identifies a novel homozygous missense mutation in exon 10 of KATNB1 where c.818G>A as a result in p.Gly273Asp change in the affected members of the family. The mutation was ruled out in 100 unrelated healthy controls. The missense homozygous mutation detected in this study has not yet been reported as pathogenic in literature or variant databases such as Exome Aggregation Consortium and 1000 Genomes project. In conclusion, the here detected homozygous missense mutation of KATNB1 gene as a results (p.Gly273Asp) alters a highly conserved residue, and the substitution is predicted to have functional effects on the protein according to the majority of in silico tools. The variant is rare in the general population based on its absence from gnomAD. This mutation identified first time in Saudi population further explain the possibility that KATNB1 gene play important role and essential for multiple aspects of normal human neurodevelopment.

**Reference:**

Toll-like receptor 4 gene expression is induced by osteogenic and adipogenic differentiation of human mesenchymal stem cells derived from bone marrow

khodabandehloo, fatemeh (1), Aflatoonian, Reza (2), Nassiri-Asl, Marjan (3), Zandieh, zahra (4), Baghaban Eslaminejad, Mohamadreza (5)

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(4) Embryologist, Iran university of medical science, Iran university of medical science, Iran.  
(5) profesor, Department of Stem Cells and Developmental Biology, Royan Institute, Iran.

Abstract:

Background: Mesenchymal stem cells from adult bone marrow can differentiate into cells of the mesodermal lineage such as osteocytes, adipocytes and chondrocytes. Previous studies indicated that MSCs express Toll-like receptors that play an important role in regulating MSC fate. Studies also have shown that Mesenchymal stem cells (MSCs) are attractive candidates for regenerative medicine. It is unknown whether differentiation of human Mesenchymal stem cells is related to alteration in toll-like receptors 4 gene. Therefore, we examined the Toll-like receptor 4 gene expression following treatment with osteogenic and adipogenic differentiation supplements in vitro. Material and methods: Human bone marrow mesenchymal stem cells were used for the aims of the study. TLR4 gene expression was assessed by Real Time PCR on days 7, 14 and 21 in trated and control groups. LPS (1µg/ml) was added to the Osteogenic and adipogenic medium to activated TLR4. The differentiation potential of MSC was evaluated in the osteogenic and adipogenic media by genes expression related to differentiation at day 7, 14, and 21. Osteogenic parameters were evaluated by measuring Ca2+deposits and alkaline phosphatase (ALP) activity. Results: Osteogenic medium (p<0.01 at days 21) and adipogenic media (p<0.05 at days 21) significantly affected TLR4 mRNA expression in comparison with control group. Lipopolysaccharide significantly increased the Toll like receptor 4 gene expression (P < 0.05 at days 7) in osteogenic group compared to with control group. There was no significant difference in Toll like receptor 4 gene expression in adipogenic group compared to with control group. Conclusion: The data from this study provide the evidence that osteogenic and adipogenic differentiation can induce Toll like receptor 4 gene expression. Alteration in Toll like receptor 4 gene expression should be considered as an essential factor in innate immune response against microbial pathogens. Therefore, TLRs can change mesenchymal stem cells fate along Transplantation. Key words: TLR4, human mesenchymal stem cells, differentiation, expression.

Attachment:

**Abstract:**

Introduction: Epithelial ovarian cancer (EOC) is one of the most lethal gynaecological cancers and has poor prognosis when diagnosed at later stages. It is estimated the nearly 22000 ovarian cases are diagnosed each year and the annual death rates are over 14000 deaths [1]. Conventional de-bulking surgery and platinum or taxane based drugs are effective in at least 70% of early diagnosed EOC. However, the major drawback with ovarian cancer prognosis is the emergence of multi-drug resistance (MDR), which leads to cancer relapse within a short span of 1 - 2 years [2, 3]. Stem cells isolated from different tissues have been reported to inhibit various human cancers both in in vitro and in vivo animal studies. Human Wharton’s jelly stem cells (hWJSCs) are derived from within the Wharton’s jelly of the umbilical cord. Being fetal in origin the hWJSCs have the properties of both embryonic stem cells (ESCs) as well as the MSCs [4]. Furthermore, unlike MSCs derived from other sources, the hWJSCs do not cause tumour in vivo in immunodeficient mice [5]. Various research groups have identified that the tumour inhibition properties of hWJSCs spans across many different human cancers [6, 7]. As such we evaluated the effects of hWJSCs on an ovarian cancer (SKOV3) cell inhibition in vitro. Materials and Methods: Ethical approval was obtained to derive hWJSCs from human umbilical cords. SKOV3 was purchased ATCC. The effects of hWJSC extracts (hWJSC-CM and hWJSC-CL) was tested initially on SKOV3 for their proliferation (MTT assay). The hWJSC-CM was tested at 12.5%, 25%, 50%, 75%, 100% and the hWJSC-CL at 5µg, 10µg, 15µg, 30µg and 50µg for 24h, 48h and 72h respectively. Standard anticancer agents doxorubicin (3nm, 10nm, 30nm, 100nm, 300nm) and paclitaxel (2.5nm, 5nm, 10nm, 20nm, 30nm) were used as positive controls. Following initial results with cell proliferation and morphological studies, we then used the effective concentrations of hWJSC-CM (50%) and hWJSC-CL (15 µg/ml) for the evaluation of cell cycle, cell migration, apoptosis, and gene expression studies. Tumour spheres (TS) were generated from SKOV3 and tested with both hWJSC-CM (50%) and hWJSC-CL (15 µg/ml) for both inhibition of TS and the expression of cancer stem cell (CSC) markers. Results: The derived hWJSCs fulfilled the minimal criteria for MSCs such as having spindle shaped fibroblastic morphology, plastic adherence and expression of MSCs related CD markers (Positive expression for CD29, CD44, CD73, CD90, CD105 and negative expression for CD34 and CD45. MTT assay showed SKOV3 inhibition following exposure to hWJSC-CM at different concentrations (12.5%, 25%, 50%, 75% and 100%) and the decreases were 20.52%, 20.52%, 22.73%, 25.86% and 31.61% at 24h; 10.34%, 15.62%, 17.07%, 21.27% and 31.67% at 48h and 14.20%, 32.81%, 42.06%, 49.03% and 65.00% at 72h (Figure 1A). The decreases obtained in SKOV3 following exposure to hWJSC-CL at different concentrations (5µg, 10µg, 15µg, 30µg and 50µg) were 12.64%, 16.82%, 22.69%, 29.03% and 43.36% at 24h; 6.45%, 12.21%, 25.48%, 35.92% and 46.35% at 48h and 2.28%, 8.25%, 29.54%, 52.02% and 55.02% at 72h (Figure 1B). The decreases in SKOV3 with higher concentrations of hWJSC-CM for 25% , 50% , 75% and 100% at 72h and hWJSC-CL (for 15µg, 30µg and 50µg at 24h, 48h & 72h) were statistically significant (p<0.05) compared to the control. Doxorubicin and paclitaxel also showed effective SKOV3 inhibition (Figure 1C, 1D). Morphological analysis SKOV3 treated with higher concentrations of hWJSC-CM (50%, 75% 100%, Figure 1E) and hWJSC-CL (15µg, 30µg and 50µg, Figure 1F) for 24h to 72h showed various changes such as cell shrinkage, membrane damage and blebbings leading to cell death. Cellular changes were both time and concentration dependent, and was more evident with hWJSC-CM (75%) and hWJSC-CL (30µg/ml) at 48h and 72h. Both paclitaxel and doxorubicin also showed time and concentration dependent morphological changes in SKOV3 leading to cell death. Cell cycle assay done on SKOV3 at 48h,
showed alteration in the cell cycle profile in both cell lines following treatment with hWJSC-CM (50%, 75% 100%) and hWJSC-CL (10µg, 15µg, 30µg) compared to the control. There was an increase in the sub G1 and G2M phases of cell cycle following treatment with hWJSC-CM (100%) and hWJSC-CL (10µg, 15µg, 30µg) indicative of cells undergoing both apoptosis and metaphase arrest respectively (Figure 1G). Migration assay done on SKOV3 following treatment with hWJSC-CM (50%) and hWJSC-CL (15µg/ml) for 48 h inhibited cancer cell migration compared to the control (Figure 1H). Annexin V-FITC assay demonstrated cancer cell apoptosis following treatment with hWJSC-CM (50%) and hWJSC-CL (15µg/ml) for 48 h (Figure 1I). The TS of SKOV3 showed changes in morphology including vacuolations, altered surface changes and reduction in the size compared to respective controls. SKOV3 stained with antibodies for CSC markers (E-Cadherin, CD117, CD133, CD90, CD31, CD34, CD44, CD47, CD309, CD24, CD105 and CD326), demonstrated the presence for some of the above CSC markers and also a mild decrease in CSCs markers expression following treatment with hWJSC-CL (15µg/ml) than hWJSC-CM (50) (Figure 1J). The gene expression showed differential expression of cell cycle regulatory genes (cyclin A2, Cyclin E1), prostaglandin receptor signaling genes (EP2, EP4) and the pro-inflammatory genes (IL-6, TNF-a). Treatment with hWJSC-CM (50%) or hWJSC-CL (15µg/ml) for 48h led to decreases in expression of the above genes compared to untreated controls (Figure 1K).

Conclusions: Both hWJSC-CM and hWJSC-CL inhibited SKOV3 cells at mild to moderate levels by inducing cellular changes leading to altered morphology, cell cycle arrest, induction of apoptosis, decreasing the expression of CSC markers, increasing or decreasing the cytokines levels and by genes regulation. Novel agents targeting important signaling pathways involved in cancer growth and metastasis, induction of apoptosis/authophagy, enhancing intracellular drug delivery and immunological agents/vaccines are being actively researched [8]. Earlier studies have identified that umbilical cord derived MSCs including the hWJSCs have inhibitory effects on various cancers either in vitro or in vivo [9, 10]. We therefore, conclude that hWJSCs and/or its extracts which are usually discarded as a medical waste can be useful adjuncts together with conventional therapies in the management of various tumours.

Reference:


Attachment:
Gauthaman et al - MEMBS -Figure 1 (A-K):
http://membs.org/membs/uploads/congress_speaker_files/1527766728Gauthaman et al - MEMBS -Figure 1 (A-K).tif
**Morphological characteristics of T47D cancer spheroids: growth comparative analysis using TEM**

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(2) Graduated, Biomedical engineering, University of Connecticut, United States.
(3) Professor, Clinical Laboratory Sciences, Jouf University, Saudi Arabia.
(4) Prof, Biomedical engineering, University of Connecticut, United States.

**Abstract:**

In this study we present the use of Transmission Electron Microscopy (TEM) to analyze 3D tumor model including morphological and cellular features. Breast cancer cell line (T47D) (ATCC, VA USA 20110) was used to develop spheroids, which provide a heterogeneous biomimetic setting for growth analysis. DMEM was used to culture T47D cells in 10% fetal bovine serum (FBS), and 1% Penicillin-Streptomycin (Waltham, MA USA 02451). The spheroid development was started by incubating them in U-bottom round-shaped well plates (Corning, NY USA 14831) under standard conditions at 37 °C with 5% CO2 in humidified incubators. Each spheroid was seeded separately with 1000 cells. In each well, cells were seeded in order to obtain spheroid with a typical diameter of 300 µm at day 5, which became about 500 µm at day 20. The tumor spheroids were collected from the channel at different time points (Day 5, Day 10, Day 15 and Day 20). An inverted microscope (Olympus, Japan 163-0914) with hemocytometer (Hausser Scientific, PA USA 19044) was used to count the number of cells. TEM (Model FEI Tecnai 12 G2 Spirit BioTWIN; FEI, OR USA 97124) was used to perform growth analysis and visualizing the cellular morphology alterations and nuclear and cytoplasmic changes of the tumor spheroid. Sample preparation of the cultured T47D breast cancer spheroids for TEM imaging and analysis was performed at the Biology EM Lab (UConn, CT USA 06269). The sample preparation steps included fixation, osmication, dehydration, embedment and ultra microtomy sectioning (ultraslicing). The TEM examination revealed marked variation in size of the cancer cells at the periphery of the spheroids. The nuclei of these cells were mostly enlarged, irregular and showed dispersed heterochromatin with enlarged nucleoli. Most of the cells in the center of the spheroids showed signs of degeneration and necrosis characterized by swelling of organelles and cytoplasmic vacuolation with presence of large vacuoles containing digested debris. The necrotic changes were observed for spheroids at day 10 and became more prominent at day 20, particularly in spheroids formed from culture containing 2000 cancer cells. Our observation in this study illustrates the potential of such analysis to serve as a reference control for analyzing 3D tumor models and for further studies related to nanoparticles interaction and mechanical characterization.

**Reference:**


**Attachment:**

Figure 1: [http://membs.org/membs/uploads/congress_speaker_files/1527773303figure.jpg](http://membs.org/membs/uploads/congress_speaker_files/1527773303figure.jpg)
Pluripotent Stem Cell-Derived Beta Cells for Type 1 Diabetes: How Far From Clinical Applications?

Ghanameh, Zain (1)

(1) Stem Cell Researcher, Research and Development, StemCellsArabia, Jordan.

Abstract:

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Abstract Recent progress in the field of regenerative therapies has focused attention on the generation of beta cells from human stem cells. Pluripotent stem cells including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are considered ideal therapeutic tools in cell replacement therapies. The most crucial factor in regenerative medicine is the development of safe and standardized protocols that can be applied to patients. Advancement of this topic is taking place in various fields, including type 1 diabetes (T1D) where the features of both hESCs and iPSCs were explored by researchers around the world showing that, with certain limitations, these cells appear to be potent for the treatment of T1D with various degrees. The discovery of new types of stem cells and development of alternative routes to pluripotency bypasses the ethical complications of ESCs widening the horizon of stem cell research and its therapeutic applications. One of the most promising approaches is the directed differentiation of human pluripotent stem cells into beta cells. Transplantation of stem cell-derived beta cells can be the most promising treatment strategy for T1D patients with impaired beta cell function. In this review, we will summarize the different strategies and protocols that have been implemented towards achieving in vitro human pluripotent stem cell-derived beta cells. Additionally, we emphasize on the emerging hurdles in T1D related pluripotent stem cell research and its clinical translation.
Identification of Spermatogenic Cells in Normal and Abnormal Semen: A Comparative Study

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(5) Clinical Assistant Professor of Regenerative Medicine, University of Illinois College of Medicine in Peoria - USA, President, Stem Cells Arabia, Jordan.

Abstract:

Spermatogenesis is a process by which male germline stem cells self-renew and differentiate to male spermatozoa. The main three stages are the mitosis of spermatogonia, meiosis of spermatocytes, and spermiogenesis. In male infertility, the spermatogenesis pathway can be arrested or disturbed for a number of reasons. Therefore, development of a specific technique for quantification of spermatogenesis and to track the progress and identify the stage, in which the arrest takes place, can have tremendous applications in treatment of male infertility. Conventional approaches based on spermatogenic cell morphology and DNA flow cytometry have been established. However, they are relatively difficult and time-consuming. Our method described here offers significant advantages for identification of spermatogenic cells including rapidity and simplicity of the method. We have developed a PCR-based method that allows us to detect any defects in spermatogenesis by identifying the three main spermatogenic cells in human semen. RNA was extracted from cells in semen samples from 50 infertile patients and 15 healthy individuals. cDNA was synthesized and was used for PCR for GPR125, GFRA1, RET, PIWIL2, PLZF, UCHL1, SCP1, SCP3, Tesmin, ACR (Acrosin), TNP1, PRM1 (Protamine 1) and PRM2 (Protamine 2) genes. The selection of these genes provides a comparative study highlighting whether analysis of these genes provides a useful non-invasive method for assessing defects in spermatogenesis. Our preliminary results showed that a significant difference was found in the types of cells found between infertile patients and healthy individuals as well as between infertile patients themselves. The method described appears to be a very good candidate as a simple, non-invasive and reliable assay for investigation of spermatogenesis in vitro and could be useful in the choice and evaluation of appropriate therapeutic as well as diagnostic strategies for male infertility patients.
Development of Fast and Scalable Protocol for Preparation of Wharton's Jelly-Derived Mesenchymal Stem Cells for Clinical Applications

Al-Zoubi, Myassar (1), Alqudah, Alex (2), Zalloum, Mahasen (3), AlBakheet, Sameh (4), Ghanameh, Zain (5), Alghadi, Ahmad (6), Yousef, Sana' (7), Al-Zoubi, Adeeb (8)

(1) Research Assistant, Research & Development, Stem Cells Arabia, Jordan.
(2) , , Stem Cells Arabia, Jordan.
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(7) Chief Research and Development Officer, Research and Development, Stem Cells Arabia, Jordan.
(8) Clinical Assistant Professor of Regenerative Medicine, University of Illinois College of Medicine in Peoria - USA, President, Stem Cells Arabia, Jordan.

Abstract:
Wharton’s Jelly-derived Mesenchymal stem cells (WJ-MSCs) have gained attention as an alternative, potent source of stem cells for regenerative medicine due to their greater self-renewal, higher proliferation capacity and differentiation potential alongside their unique immunomodulatory properties. However, differences in the quality and functionality properties of the final prepared MSCs are highly dependent on the preparation and expansion methods. Current protocols to prepare WJ-MSCs yield low numbers of cells with variable proliferation potentials. Therefore, a need arose toward standardizing efficient and scalable preparation protocols. In this study, we have successfully prepared WJ-MSCs by using our enhanced explant-based protocol that yields a high number of cells with relative ease, simplicity, and speed. The prepared cells were then examined for their pluripotency, immunophenotypes, and differentiation capacities. Expression of pluripotency and fibroblast markers: IDO, TGF?, OCT1, OCT2, OCT4A, OCT4B, NANOG, SOX2, FAP, and FSP were analyzed using qRT-PCR. Additionally, immunophenotypic characterization of prepared WJ-MSCs was analyzed by flow cytometry against CD105, CD73, CD45, CD133, CD117, CD90, CD34, CD49, CD146, MSCA1, SUSD2, and SSEA4 surface markers. Furthermore, the prepared cells were induced for differentiation into adipocytes, chondrocytes, and osteocytes. Our results showed a high proliferation capacity of WJ-MSCs. Immunophenotyping further confirmed the expression of MSC markers of the prepared WJ-MSCs. Furthermore, they demonstrated noticeable differentiation potential into the tri-lineage. The present study has revealed the feasibility of our fast, efficient and scalable protocol; however additional qualitative and quantitative studies are warranted to translate the use of WJ-MSCs for clinical applications to ensure safety and efficacy of the prepared cellular products.
**Metagenomics Analysis of UAE and Indian Population using Next Generation Sequencing**

Abdelaal, Lamis (1), Shahid, Muhammad (2), Alsennani, Salima (3)

(1) Assistant Professor, Forensic biotechnology, UMS, United Arab Emirates.
(2) Assistant Professor, Biotechnology, University of Modern Sciences, Dubai, United Arab Emirates.
(3) Student, Forensic biotechnology, UMS, United Arab Emirates.

**Abstract:**

Metagenomics study emphasize the DNA analysis of microbiome using 16S rRNA gene. This gene is encoding the RNA component of the smaller subunit of bacterial ribosome and contains hypervariable regions that gives the species a specific sequence signature to be used in identification of bacteria. Microbial DNA of microbiome is a collection of different microorganisms which are present in contact with human surfaces either internally or externally; which could be helpful in producing evidence admissible in court. Forensic microbiology in conjunction with forensic anthropology can be used to trace individuals living in specific area and to connect the suspect with victim. The present research will focus on the Metagenomics analysis of hair samples of Emirati and Indian population. About 150 hair samples of both males and females will be collected using hair collection kit from Emirati and Indian populations living in UAE. Both the age group (15 -30 years of age) and environmental factors will be consist to achieve uniform results. DNA extraction will be performed using hair DNA extraction kit (Applied Biosystems). DNA quantification will be carried out using Real time PCR machine. 16S rRNA gene will be amplified using specific PCR primers. The amplified PCR products will be sequenced using next generation sequencing platform. The result will be analyzed using bioinformatics and statistical tools. This research work will be very useful for forensic investigations and case work analysis and also to differentiate UAE and Indian population using microbiome expression analysis results.

**Reference:**

Peters Plus Syndrome: Molecular analysis of B3GALTL gene in 11 Tunisian cases

Lajmi, Yosra (1), Kanoun, Houda (2), Guirat, Manel (3), Gharbi, Nourhene (4), Siala, Olfa (5), Chabchoub, Imen (6), Hmida, Nedia (7), Hachicha, Mongia (8), Fakhfakh, Faiza (9), Gargouri, Abdellatif (10), Ammar Keskes, Leila (11), Kammoun, Hassen (12), Belguith, Neila (13)

Abstract:

Introduction: Peters plus syndrome is an autosomal recessive rare disorder comprising ocular anterior segment dysgenesis, short stature, hand abnormalities, distinctive facial features and mental retardation. It’s related to mutations in the B3GALTL gene, leading to the inactivation of the B1, 3-glucosyltransferase. Up to now, 13 mutations in the B3GALTL gene were identified in patients with PPS. In Tunisia, the homozygous c.597-2A>G mutation, identified in the exon 8 of the B3GALTL gene, was the first molecular diagnosis of PPS. Materials and methods: In order to confirm the diagnosis of Peters plus syndrome and to develop genetic counseling, we studied 11 Tunisian patients belonging to 6 unrelated families. 9 patients have clinical features that strongly suggest PPS and 2 patients consulted for prenuptial genetic counseling. For our patients, the Genomic DNA was isolated from whole blood and DNA extraction from blood leukocytes was performed followed by a search for the c.597-2A>G mutation by PCR / RFLP. If the c.597-2A>G mutation wasn’t found, the sequencing of the whole gene was performed. Results and discussion: The results revealed the presence of a homozygous A to G substitution at position ?2 of the acceptor splice site of exon 8 (c.597-2A>G) in 7 of the 9 patients having the clinical features of PPS (P1, P2, P3, P4, P5, P6 and P7). The functional study showed that this mutation modified the splice acceptor site of exon 8 of the B3GALTL gene leading to a total jump of this exon. The absence of exon 8 induces a shift of the reading frame and the premature introduction of a stop codon within exon 9 marking the end of gene translation. The result is the formation of a truncated protein devoid of its catalytic domain. For the other two patients having the PPS (P8 and P9), a heterozygous c.597-2A>G mutation was found in one patient (P8) whereas, the mutation was absent in the other patient (P9). So we have completed the sequencing of the entire gene B3GALTL for these 2 patients. A new intron mutation was identified in the exon 4. This mutation was identified in the heterozygous composite state with c.597-2A>G in patient (P8) and in the homozygous state in patient (P9). Finally, for the two patients consulting for prenuptial genetic counseling (P10 and P11), the c.597-2A>G mutation was identified in the heterozygous state for both of them, the risk of recurrence is 25%, a prenatal diagnosis was proposed in case of pregnancy. Conclusion: In summary, our study shows the importance of genetics by reporting two mutations in the B3GALTL gene responsible of PPS in Tunisian patients, providing the families with the opportunity for prenatal molecular diagnosis in future pregnancies for an accurate prenatal diagnosis. Abreviations: PPS:
Peters Plus Syndrome P: Patient

**Reference:**

**Attachment:**
Fig 1: genealogical tree:
http://membs.org/membs/uploads/congress_speaker_files/1527786081genealogical tree.png

mutation:
Assessment of Sequestosome 1 mutation in Tunisian Paget bone disease

Frikha, R. (1), Zouinkhi, Wided (2), Frikha, Rim (3), Ghozzi, H. (4)

(1) Associate Professor in molecular genetics, Laboratory of Histology, Faculty of Medicine of Sfax, Tunisia.
(2) PhD student, Laboratory of Histology, University of Sfax, Tunisia.
(3) Associate Professor in molecular genetics, Medical Genetics and Reproductive department, Hospital University Hedi Chaker, Sfax-Tunisia, Tunisia.
(4) Associate Professor in pharmacology, Laboratory of pharmacology, Faculty of Medicine of Sfax, Tunisia.

Abstract:

Background: Paget bone disease (PBD) is a common skeletal disorder with a strong genetic component, which is characterized by focal increases in disorganized bone remodeling, predominantly affecting the axial skeleton. Mutations of Sequestosome 1 (SQSTM1) are the most common cause of classical PBD. Currently, several mutations of the SQSTM1 gene have been described and the P392L is the most prevalent affecting the ubiquitin-associated domain (UBA) of the SQSTM1 protein. The aim of this study was to assess the P392L mutation in Tunisian Paget Bone disease, to establish the correlation between phenotype and genotype.

Methods: Peripheral blood (PB) samples from 11 patients were obtained with a referring diagnosis of Paget disease. Genomic DNA was extracted from EDTA-anticoagulant blood samples according to the salting protocol. A polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP) was applied for detection of the SQSTM1 mutation (P392L).

Results: Among PBD patients, 8 cases were heterozygous for P392L of SQSTM1 with an overall frequency of 72.7%. A particular history of complications was recorded in these affected patients.

Conclusion: To the best of my knowledge, this is the first study carried in Tunisian PBD, and the frequency is higher than reported previously [1, 2]. The P392L of SQSTR1 seem to be associated with a severe phenotype. Larger studies are mandatory to draw a conclusion.

Reference:

Metagenomics Analysis of UAE and Indian Population using Next generation sequencing

Shahid, Muhammad (1), Alsennani, Salima (2), Abdelaal, Lamis (3)

(1) Assistant Professor, Biotechnology, University of Modern Sciences, Dubai, United Arab Emirates.
(2) Student, Forensic biotechnology, UMS, United Arab Emirates.
(3) , , UMS, United Arab Emirates.

Abstract:
Metagenomics study emphasize the DNA analysis of microbiome using 16S rRNA gene. This gene is encoding the RNA component of the smaller subunit of bacterial ribosome and contains hypervariable regions that gives the species a specific sequence signature to be used in identification of bacteria. Microbial DNA of microbiome is a collection of different microorganisms which are present in contact with human surfaces either internally or externally; which could be helpful in producing evidence admissible in court. Forensic microbiology in conjunction with forensic anthropology can be used to trace individuals living in specific area and to connect the suspect with victim. The present research will focus on the Metagenomics analysis of hair samples of Emirati and Indian population. About 150 hair samples of both males and females will be collected using hair collection kit from Emirati and Indian populations living in UAE. Both the age group (15 -30 years of age) and environmental factors will be consist to achieve uniform results. DNA extraction will be performed using hair DNA extraction kit (Applied Biosystems). DNA quantification will be carried out using Real time PCR machine. 16S rRNA gene will be amplified using specific PCR primers. The amplified PCR products will be sequenced using next generation sequencing platform. The result will be analyzed using bioinformatics and statistical tools. This research work will be very useful for forensic investigations and case work analysis and also to differentiate UAE and Indian population using microbiome expression analysis results.

Reference:

Attachment:
Salima Metagenomics 2018:
A Sensitive Molecular Method for Nucleic Acid Discovery

alshaikh, sana (1)

(1) Consultant Molecular Virology, Molecular Biology Lab , Ministry of health, Saudi Arabia.

Abstract:
Recent sequence independent amplification (SIA) methods for viral discovery have proved successful in detecting new viruses in many biological samples other than cerebrospinal fluid (CSF) samples. A known problem with these assays is the annealing of the random primers used to human DNA which facilitates preferential amplification of background DNA. Thus, large scale sequencing is usually required to detect a virus, which in turn reduces the detection sensitivity to more than 1000 copies/µl, a CSF concentration that is rarely seen in cases of viral encephalitis. We present here a highly sensitive SIA assay that could be used in testing CSF samples obtained from patients with encephalitis. We started with evaluation of two SIA assays commonly used for virus discover. Sequential modification and adaptation of these methods was carried out to increase their sensitivity. Ultimately, a novel primer (Sa primer) that showed no binding to most human DNA sequences in GenBank was designed. Its 3’ end was tagged with 6 and 7 random nucleotides generating 2 r-primers; Sa-6 and Sa-7. The sensitivity of the r-primers was checked in a novel assay developed during this project and named Sa-SIA using known concentrations of human cytomegalovirus and human herpes virus type 1.
Molecular characterization of Campylobacter jejuni and its relation to invasion and clinical infection

alshaikh, sana (1)

(1) Consultant Molecular Virology, Molecular Biology Lab, Ministry of Health, Saudi Arabia.

Abstract:

Toxin production and invasion of epithelial cells are mechanisms which have been identified in the pathogenesis of Campylobacter diarrhea. Various virulence genes associated with these pathologic processes have also been identified. However, the correlation between the presence of these genes in clinical isolates, in-vitro invasive capability and in-vivo severity of infection (in the patients they were isolated from) remains to be clearly described. In this study, we have investigated the molecular characterization of C. jejuni strains isolated in the Kingdom of Bahrain and correlated these with their invasive capabilities and patient’s clinical presentations. Eight C. jejuni strains isolated from patients in the Kingdom of Bahrain were studied. Clinical data of the patients was obtained and scored to determine the severity of infection. Polymerase chain reaction (PCR) was carried out to determine the presence of cytolethal distending toxin (cdtB), invasion associated marker (iam) and Campylobacter invasion antigen (ciaB) genes. Adherence and invasion assays in both INT 407 and HeLa cell lines were carried out using standard methods. The pattern of invasion observed was similar in both cell lines. Two strains positive for all three genes showed the highest level of invasion and were isolated from patients with the most severe clinical presentation. Two cdtB+ve/ciaB+ve, two cdtB+ve/iam+ve and two strains negative for all three genes were identified. Strains positive for both cdtB and ciaB genes were more invasive and associated with more severe clinical presentation compared to cdtB+ve/iam+ve strains. One of the two strains negative for all three genes showed invasion levels and clinical severity similar to cdtB+ve/ciaB+ve strains. However, the second strain negative for all three genes showed non-invasive phenotype with mildest clinical presentation. These findings suggest a correlation between in-vitro invasive capability and clinical presentation. The presence of all three genes was associated with higher degree of invasive capability and clinical infection. However, their absence did not preclude virulence, suggesting a role for other genes or factors. Although the presence of ciaB gene was associated with higher degree of invasiveness and worse clinical presentation when compared to iam, further work is needed to confirm this finding. Further collaborative work on characterizing the clinical C. jejuni isolates in the GCC is needed to enable a clearer understanding at the molecular level of the pathogenic features of the strains circulating in this region.
Identification of new ATII cells surface markers as target of siRNA delivery by Bispecific antibodies (bsAbs) for lung diseases therapy

Hasan, Diya (1)
(1) Assistant Professor, Allied Medical Sciences, Al Balqa Applied University, Jordan.

Abstract:
Lung diseases are common medical condition that is considered as a leading cause of death worldwide. Common therapies of lung diseases such as pulmonary fibrosis, lung cancer and inflammatory have insufficient efficacy. Small interfering RNA (siRNA) provide a new and strong approach to treat or prevent several diseases including respiratory ones by regulating gene expression. It presents various advantages over the traditional ones such as protein and small molecules based drugs. They could effectively target any genes selectivity, in addition to their simple design and synthesis since they do not require a refolding schemes or cellular expression system. Regardless its enormous therapeutic potential, delivery remains to be a crucial barrier to the clinical application of RNAi therapeutics. Besides, siRNA systemic distribution usually requires very high doses which lead to its distribution in untargeted locations, inducing side effects such as immune response. Bispecific antibodies (bsAbs) are able to recognize and bind to cell surface antigens so they can be implied for targeted siRNA delivery. Alveolar type II cell (ATII) covers a small area of the alveolar surface and they secret pulmonary surfactant, which reduces surface tension in the alveoli. In the present work we investigated potential ATII cell specific membrane proteins which can be used as therapeutic target for bsAbs in a number of common lung disorders. Our immune staining results of mouse lung tissues confirms ATII cells membrane surface markers ITGB2 and ITGB6 as diagnostic and therapeutic targets. Interestingly we had observed some co-lonilized areas of SFTPC (surfactant associated protein C) with new candidate protiens including: PTPRC and ANPEP. The enrichemnt % of ANPEP at the ATII cells membrane was higher than PTPRC. Our findings identify PTPRC and ANPEP as a new protein markers at ATII cells membrane surface. They represent attractive target to be a main player in our project future steps for siRNA delivery by bsAbs.
Transcriptome profiling of thermophile plant Heliotropium thermophilum

Kadioglu, Asim (1), Saglam, Aykut (2)

(1) professor, biology, Karadeniz Technical University, Turkey.
(2) Associate Professor, Molecular Biology and Genetics, Karadeniz Technical University, Turkey.

Abstract:
In this study, the transcriptome profile of Heliotropium thermophilum plant adapted to live at high temperature was obtained. For the study, the plants living at 30 degree celsius (control group) and 60 degree celsius were compared. total RNA was isolated from the leaves of the plants and sequenced with NGS. According to the results, it was determined that over 500 genetic levels of the individual living at 60 degree celsius level increased statistically significantly compared to the control group. high temperature has altered carbohydrate metabolism, ion balance and osmolite synthesis. thus the H. thermophilum plant is believed to be adapted to the high temperature.
Serum Osteocalcin Concentration and Its Association with Some Clinical Parameters in Diabetes Mellitus

Saeed, Liqaa (1), Ali, Thikra (2)

(1) Lecturer, Biochemistry, universcity of mosul, Iraq. (2) professor , Chemistry, biochemistry, Iraq.

Abstract:

The research included estimate the concentration of osteocalcin and some clinical parameters in control and diabetic patients (Type I and Type II). The results demonstrated that the normal mean of osteocalcin in serum was (28.07 ± 4.79 ng/mL) in control group. also, the results demonstrated a significant decrease in the concentration of osteocalcin in serum of type I and type II diabetic patients compared with control and between type I diabetic patients compared with type II. The results also showed a significant increase in the concentration all from glucose, trehalase, MDA, total lipids, total cholesterol, triglyceride, VLDL-C, LDL-C, atherogenic Index, potassium in serum of diabetic patients(type I and II), while found a significant decrease in the concentration all from insulin, HOMA-IR, thioredoxin, in serum of diabetic patients (type I) compared with control and diabetic patients(type II), and a significant decrease in the concentration all from GSH, HDL-C, Antiatherogenic Index, sodium, calcium, magnesium, and zinc in serum of diabetic patients (type I and II) compared with control. Also, the results showed a significant increase of insulin resistance in type II diabetic patients compared with control and type I diabetic patients, and a significant increase of adiponectin concentration between type I diabetic patient compared with type II and control. Correlation coefficients between osteocalcin and some clinical parameters of control and diabetic patients showed that osteocalcin concentration has a significant negative correlation with concentration all from glucose, HOMA-IR, trehalase, total cholesterol, triglycerides, VLDL, LDL-C, and atherogenic Index, and a significant positive correlation with concentration all from HOMA-?, thioredoxin, GSH, HDL, antiatherogenic Index, sodium, calcium, and zinc in control and diabetic patients(type I and II). While osteocalcin has a significant negative correlation with concentration all from MDA, total lipids, and a significant positive correlation with concentration all from insulin, adiponectin, magnesium in control and type II diabetic patients, whereas osteocalcin has a significant negative correlation with potassium concentration in type II diabetic patient.

Reference:


Patho-genetic study of vincristine effects on spermatogenesis and ovulation in mice.

sachit, muna (1), Abbas Abdul-Majeed, Ban (2), sachit, zainab (3), Youis, Eman (4)

(1) student, Molecular Pathology and Genetics, College of veterinary medicine , Iraq.
(2) Consultant Molecular Pathology and Genetics, Al Nahrain University, Iraq.
(3) consultant, Molecular Pathology, college of medicine kufa university, Iraq.
(4) , , Vet.medicine, Iraq.

Abstract:
Background & Objective: Many chemical drugs which are plants' origin such as vincristine are interfering with fertilization and reproduction. Florescence in situ hybridization for mouse molecular cytagenetic are emerging including the definition of transgenic integration sites in epigenetic studies. Detection of nucleotide sequences on examined DNA molecule consists of hybridizing DNA probe to its complementary sequence on chromosomal preparation. Hybridization is formation of a duplex between the complementary (single stranded) sequences of nucleic acid. Methodology: The study conducting on one hundred (100) mixed mouse were kept in animals' house in college of Veterinary Medicine in Baghdad University and fed on special pellet and drank on tap water in special bottles, divided to three group, 1st group 40 male were treated with vincristine sulfate intraperitoneal at dose (0.1mg/10gm body weight) for 8th week, 2nd group 40 female were treated with vincristine sulfate intraperitoneal at dose (0.1mg/10gm body weight) weekly for 8th week. 3rd group 20 mixed mouse consider as control treated with distilled water Results: 1- Genetic examination results showed evident of DNA damage in TK (11qE2)/XY gene on testicular and ovarian tissues, this damage was diagnosed by using florescence microscopy after application of FITC procedure. The differences between green signals and red signals indicated the defect in DNA , results showed low differences between red and green signals in control group and accrued at at score 1 (0-0.5%) no or low percent of DNA defect because there were two copy of red signals beside two copy of green signals in over 7 field of examination from 10 field , while in experimental groups reported mainly within score 4(40-95%) and score 5(<95%) within high percent of DNA defect, which showed differences between red and green signals because there were losing in equality of double copy of signals such as there were 3 red signals beside 1 green signals that referring to deletion as well as 5 green signals beside 2 red signals referring to amplification and chromosomal fragmentation appear as yellowish signals in more than 8 field in each 10 field of examination . 2-Pathological examination showed testicular and ovarian tissues hypoplasia with vaculated and necrotic lesion on seminiferous tubules and epididymis, loss of spermatogenesis and ovulation after 4th week of treatment with vincristine sulfate. Conclusion: vincristine has patho-genotoxic effects on mouse's spermatogenesis and ovulation. Key words: Genetic analysis; FISH ; Mouse.

Reference:

Attachment: discussion:
http://membs.org/membs/uploads/congresspeaker_files/1527805205Discussion.docx
**Curcuma Longa Extract as a Histological Dye for Renal Tissues**

**IBNOUF MOHAMED AHMED, ABD ALHAFEEZ OSMAN (1)**

(1) Port Sudan , Histopathology and cytology, Port Sudan Ahlia College, Sudan.

**Abstract:**

Background and Objectives: with the worldwide concern over the use of ecofriendly and biodegradable materials, the use of natural dyes obtained from plants has again gained interest. This experimental descriptive study aimed to find out the degree of quality of staining of renal tissues by curcuma longa solution compared to H&E routine stain. Methods: normal tissues were obtained from a kidney of a healthy rabbit and stained Using Curcuma Longa solution instead of eosin in the different Hematoxylin-eosin protocols. Results: The best results were obtained with the time duration 1-5 and 10 -20 min when using either Harri's or Weiger's Hematoxylin, while staining by Mayer's Hematoxylin mostly gave v.good results. Interpretation and Conclusions: Curcuma longa extract is a promising histological dye that effectively can replace Eosin stain in the Hematoxylin & Eosin staining protocol for renal tissues.

**Reference:**


**Attachment:**

Curcuma Longa Extract as a Histological Dye for Renal Tissues :

Diseases, Biotechnology, Microbiology, Virology, Infectious Diseases

**Development New Multiplex R T- Q RTPCR to Detect Human Norovirus Genogroups I and II**

Mohamed, Nadira (1), Nadhir , Mohammed (2), Naji, Shaymaa (3)

(1) Head Of department, gene bank and genetic sequence, Forensic DNA research and training center/Al-Nahrain university, Iraq.
(2) Head of Department of Biology, , Department of Biology, Faculty of Education, Tikrit University, Iraq.
(3) lecturer, biology, Faculty of Education / University Samarra, Iraq.

**Abstract:**

Globally, one out of every five cases of acute gastroenteritis was caused by Norovirus which is consider a highly contagious virus due to the increase in the number of cases of the virus in recent years. Therefore, there is a need to develop rapid, specific and sensitive detection and diagnosis methods. Genetic analysis is an important factor in diagnostic tests of pathogens. Using the data from the National Center of Bioinformatics Information (NCBI), the sequence of more than 1000 Norovirus genogroup II genome and 39 genome of genogroup I were chosen To select a common conserve region for all genotypes within the first group and the second group suitable for the design of primers and Probes. The design of the first area of the open junction frame (ORF) and the open reading frame two (ORF2), and the primers and probes were tested on samples of compared to the primers and mentioned in( Lazario 2010) using one step reverse transcription real time PCR. The kit designed for multiplex reverse reaction possess no overlap or interaction in the components of primers and probes of the two genogroups, also it is superior on the monoplex mixture of the previous kit, Norovirus being diagnosed in 45% of samples by primers and probes designed in this study, 10% of the samples were belong to the genogroup I and 35% of the samples belong to the genogroupII in comparing with virus results that tested with monoplex previous primers and probes accounted 44%, the genogroup represented 10% and genogroup II represented 34% of the tested samples. The use of the multiplex kit contributes to shortening the time and the largest number of samples and reduce amount of the solutions used, more than the monoplex reaction, as it should be performed separately for each genetic group.

**Reference:**

Abstract:

The study include the effect of Equisetum arvense and Urtica piluifera extracts dissolved in cold and hot water, on Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities of Leishmania tropica promastigote. The inhibitory concentration of 50% of the promastigotes (IC50) at the log phase (96) hrs was 1.57g / ml of Equisetum arvense and 1. 57g / ml of Urtica piluifera extracts dissolved in cold and hot water. The results revealed that these extracts were studied has inhibitory effect of L. tropica. promastigotes number, the number of L. tropica reduced gradually when using 0.5 to 2.5 g/ml concentrations of extracts. Moreover, these extracts had inhibitory effect on Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities. The activity levels of (GOT) and (GPT) enzymes were estimated in L. tropica promastigote which had been exposed to 1.57g / ml and 1. 57g / ml concentrations of the E. arvense and U. piluifera extracts dissolved in cold and hot water after 96hrs. of cultivation. Based on the results obtained, the changes in GOT and GPT levels in L. tropica Promastigote suggested that L. tropica showed adaptive elevation in the activity levels of the two aminotransferase enzymes in the parasite thereby probably aiding gluconeogenesis through transamination of glucogenic amino acids to meet the energy demand under extracts effect. The results shows that the GOT enzyme activity decreased by 1.8 % after exposure to E. arvense extract after 96hrs. While were 1.8 % and 3 % after exposure to U. piluifera extracts dissolved in cold and hot water. The GPT enzyme activity of L. tropica decreased by 36.4 % after exposed to E. arvense extract after 96hrs. While were 45.5 % and 53.9 % after exposure to U. piluifera extracts dissolved in cold and hot water. The results of chemical analysis of the plants has highly effect on total protein, and lees effect on total carbohydrates of cell membrane. Moreover, the extract was effect on total Nucleic acids of Leishmania tropica promastigotes after 96hrs. of cultivation.

Reference:


Attachment:

http://membs.org/membs/uploads/congress_speaker_files/1527856574Tables.docx
The Expression of Retinal micro RNA Evoked by Hyperglycemia and after Adiponectin Treatment in Human Retinal Endothelial Cells

Al-Sadeq, Duaa (1), Rizk, Nasser (2)

(1) PhD Student and researcher , Genomics and Precision Medicine, Hamad Bin Khalifa University , Qatar. (2) Associate professor, Biomedical Since Department, College of Health Sciences - Qatar University, Qatar.

Abstract:

Background: Diabetes mellitus is a chronic metabolic disease resulting in microvascular complications including diabetic retinopathy (DR). Adiponectin (ApN) is an adipokine hormone, and recent studies demonstrated that ApN could ameliorate critical biological process involved in the pathogenesis of DR. Micro-Ribonucleic acids (miRNAs) have been documented as novel biomarkers and are increasingly considered as molecules with significant modulatory action. Aim: To characterize the miRNA profile and expression in human retinal endothelial cells (HRECs) exposed to hyperglycemic conditions (HG) and illustrate the effect of adiponectin on miRNA expression and related pathways in HRECs exposed to HG. Methods: HRECs were treated with high glucose (30mM) for 96 hours duration followed by adiponectin (30µg/ml) for 24 h. Total RNA was extracted from HRECs. The gene panel array for both adhesion and angiogenesis molecules were performed using commercial RT2 Profiler PCR arrays. Furthermore, we utilized the small RNA sequencing for microRNA expression profiling of the HRECs. Results: HG treatment increases the expression of different well-known adhesion and angiogenesis genes as well as predicted miRNAs involved in these pathways, which was counteracted by ApN. RNA-Seq for miRNA profiling revealed 13 differentially expressed miRNAs in HRECs exposed to HG. miR-146a-5p was differentially expressed in HRECs treated with ApN. Analysis pathway linked the significantly changed miRNAs induced by HG to essential pathways such as hypoxia signaling, inflammation, and oxidative stress. Conclusion: HG induces expression of various adhesion and angiogenesis genes. Using RNA-Seq technology can accurately identify dysregulated miRNA profiles in HG retinal cells. MiR-146a was upregulated by adiponectin which targets different pathways involved in DR genesis.

Reference:

11. WHO. World health report 2013: Research for universal health coverage 2017 [Available from: http://www.who.int/hr/hr/en/].


The expression of retinal micro RNA evoked by hyperglycemia and after adiponectin treatment in human retinal endothelial cells.

Abstract:

Breast cancer is one of the most common and heterogenous cancer types, and the first cause of death related to cancer in women. Breast cancer is triggered by endogenous and/or exogenous factors. These factors lead to critical mutations and/or epimutations in important genes including oncogenes and tumor suppresser genes. These genetic and epigenetic changes lead to cancer initiation and cancer progression. During these processes, cells gain alteration and dysregulation in gene expression at different levels. The most important and critical level of gene expression alteration in cancer is the transcriptional level. Our current project is part of a larger project, in which we hypothesized that breast cancer transformation might have common transcriptional reprogramming events, that are associated with misregulation in gene expression, which reflects on cellular activity and homeostasis. In this part of the project, the aim was to test the ability to generate a proof of concept breast cancer transformation model that can be used to study transcriptional reprogramming in breast cancer and identify specific TFs that can be used as biomarkers for diagnosis, prognosis or even treatment of breast cancer. An in vitro breast cancer transformation model using HRAS overexpression in immortalized non transformed normal epithelial mammary gland cells (MCF10A) was generated. After HRAS overexpression, different cell phenotypes were tested, known to be induced by HRAS overexpression, that in order to ensure successful transformation. the transformed cells were then tested (not by us in this part of the project), for transcriptional reprogramming. Using different techniques, the model, indeed, showed massive genome wide transcriptional re-programming. Among the different transcription site activities that were lost are transcription sites of p53 and p63. In order to evaluate the role of these transcription factors in this transformation model, the functions of these two important TFs (p53 by using Nutlin-3a, and p63 by its overexpression) were reactivated. Our results, showed that the induction of these TF functions was enough to revert to a certain extent some the transformation process-related phenotypes. In conclusion, our transformation model can be used as an efficient tool to learn about transcriptional re-programing during cellular transformation, to identify and study the role of specific TFs in transformation. This may contribute to identifying some target genes involved in breast carcinogenesis and employ them in prognosis, diagnosis and treatment.

Reference:

Arginase Enzyme Activity and Lactoferrin Protein Concentration in Egyptian Diabetic Patients.

Shams Elddin, Nashwa (1), A. El-Desouky, Mohamed (2)

(1) School of Biotechnology and Biomolecular Sciences, Department of Chemistry, Lab of Biochemistry, Faculty of Science, Cairo University, Egypt.
(2) Assistant Professor, Department of Chemistry, Lab of Biochemistry, Faculaty of Science, Cairo University, Egypt.

Abstract:

Purpose: This work has been carried out to evaluate the arginase activity and lactoferrin (LF) level as biochemical markers in type 2 diabetic Egyptian. Subjects and Methods: The present study consisted of 84 patients classified into three groups: Uncontrolled diabetic patients [G1], Control diabetic patients [G2], Normal healthy subjects [G3]. Lactoferrin concentration was measured using ELISA technique. Arginase and other biochemical parameters were measured by using Jenway 6105 uv/vis spectrophotometer. Finally all the results were statistically analyzed and compared with normal subjects [G3]. Results: The mean sera value of both arginase and LF was found to be elevated in uncontrolled type 2 diabetic patient group [G1] and controlled type 2 diabetic patient group[G2] compared to normal healthy group [G3]. Conclusion: Both arginase and LF are considered as good biomarkers for diagnosis of type 2 diabetes and for detection of the best and most effective method for the treatment. Keywords: (1) type 2 diabetes (2) Arginase (3) Lactoferrin, LF

Reference:


Attachment:
Published Paper:
A multiplex PCR technique to characterize human umbilical cord derived mesenchymal stem cells

mohammad, Mohammad (1), Hassan, Ghmkin (2)

(1) Companies Coordinator, Coordination Department, Middle East Molecular Biology Sources - MEMBS, Syria.
(2) Master Student, Department of biochemistry and microbiology, School of Pharmacy, University of Damascus, Syria.

Abstract:
Mesenchymal stromal cells (MSCs) have the potential for self-renewal, immunomodulation, and differentiation into mesoderm lineages in vitro and in vivo. Currently, MSCs are considered as an important resource in regard to regenerative medicine applications. They can be isolated from various tissue types, including bone marrow (BM), lung, fat, liver, cord blood, amniotic fluid, placenta, and umbilical cord. Human mesenchymal stem cells (MSCs), with capacity to differentiate into adipocytes, osteoblasts and chondrocytes, were analyzed for the mesenchymal phenotype and differentiation ability using a multi-marker PCR with four primer sets specific for CD44, CD90, CD105, and GAPDH allowing a gel-based differential detection of the PCR products. To determine degree of variability of MSCs populations in terms of proliferation, cell proliferation assays were performed on expanded MSCs up to the five passage. Our results suggest that decrease in the expression of MSCs specific markers correlates with down-regulation of proliferation ability and differentiation efficiency of MSCs.

Reference:

Attachment:
SIP1/ZEB2 INDUCED EPITHELIAL MESENCHYMAL TRANSITION PROMOTES METASTASIS AND ALTERS CHEMOKINE (C-C MOTIF) LIGAND 5 EXPRESSION TO MODULATE TUMOUR MICROENVIRONMENT IN COLORECTAL CANCER

Al saihiati, Hajir (1)

(1) Assistant Professor, college of applied medical sciences, University of Hafr Albatin, Saudi Arabia.

Abstract:

Epithelial mesenchymal transition (EMT) is a critical trans-differentiation program driving cancer metastasis. Patients showing signs of EMT or presence of distant metastasis have poor prognosis. Another well-known feature of decreased cancer-associated survival is the lack of anti-cancer immune responses. Thus I hypothesized that EMT and anti-tumor response could be linked via altered secretion of soluble factors by metastatic cells. Colorectal cancer (CRC) cell lines and SIP1 inducible CRC cells were grown in DMEM. The induction of SIP1 gene was carried out using doxycycline for 3 days. EMT status of CRC cell lines were assessed by preforming western blotting, immunofluorescence and RT-PCR for EMT biomarkers. Cytokine/chemokine expression in SIP1 inducible CRC cells was analyzed using R&D systems antibody arrays. Validation of the selected CCL5 has been done using CCL5/RANTES sandwich ELISA as well as RT-PCR, and then the CCL5 expression level was analysed in CRC cell line panel by the same techniques. CCL5 promoter was cloned into pGL3. The mechanism of action of ZEB1/2 on CCL5 promoter was studied by luciferase assay and ChIP. CCL5 coding region was cloned into pcDNA3.1 and stably transfected into DLD-1 cells. DLD-1 cells overexpressing CCL5/RANTES were injected orthotopically into SCID mice, and metastasis was investigated by immunohistochemistry (IHC). T lymphocytes (TILs) infiltration in respect to CCL5/RANTES and SIP1 expression was studied in 75 CRC patients by IHC and tissue microarray. The results of EMT status catargorised 13 CRC cell lines as epithelial, intermediate epithelial, intermediate mesenchymal and mesenchymal. Cytokine/chemokine antibody arrays showed a significant increase in CCL5/RANTES in induced CRC-SIP1 cells. ELISA, Multiplex assays and RT-PCR confirmed the increase abundance of secreted CCL5/RANTES in the induced DLD-SIP1 cells. The CRC cell line panel showed that the average secreted CCL5/RANTES from mesenchymal CRC cells is significantly more than epithelial ones (107.6 ± 30 vs 639.7 ± 175 pg/ml) with a p =0.0075. An mRNA expression profiling confirmed this finding of the CRC panel. Promoter studies showed that ZEB1/2 bind to CCL5 promoter and thus activate CCL5 gene expression. No metastasis was observed for DLD-1 cells overexpressing CCL5/RANTES when orthotopically injected into SCID mice. Our data shows that CCL5/RANTES is up-regulated by EMT inducing transcription factor SIP-1, and mesenchymal (metastatic) CRC cells secrete significantly more CCL5/RANTES compared to epithelial (non-metastatic) ones. Furthermore, abundant secretion of CCL5/RANTES can be a crucial regulator of immune infiltrate in CRC, but not a direct inducer of metastasis, and that needs to be investigated. Inhibiting CCL5 activity in metastatic CRC may have a therapeutic potential.
Evaluation of the Effectiveness of Natural Product Nanoparticles and Phytoestrogens as Adjunct Agents for Treating Breast Cancer, In Vitro

Klaab, Zeinab (1), AlMalki, Faizah (2), Hassan, Aziza (3)

(1) Master Student, Human Genetics, Taif University, Saudi Arabia.
(2) Assistant Professor, Biotechnology Department, Taif University, Saudi Arabia.
(3) Professor of Biotechnology, Biotechnology Department, Taif University, Saudi Arabia.

Abstract:
INTRODUCTION Cancer is one of the most deadly diseases in the world and the number of new cases increases every day. Breast cancer in females is the most common diagnosed cancer and the second leading cause of cancer death. Despite the advances in treatments, the overall survival rate from breast cancer has not been improved substantially over the past 30 years. So, there is a need to develop novel approaches for therapies based on the targeting of cancer cells. Plants derived compounds, play a pivotal role in controlling hemostasis, by having potent antioxidant and free radical scavenging properties. However, these natural products when used with entities in nanometer sizes enable to solve many of the inherent problems (stability, solubility and toxicity) associated with natural products, and also provide a platform for targeted delivery to tumor sites. Hence, these natural compounds together with chemotherapeutic drugs improve the efficacy of these agents in induction of apoptosis in cancer cells and overcome the problem of drug resistance. Ziziphus jujube (Ziziphus) plant has exhibited phytoestrogenic properties and has numerous medicinal and pharmacological properties including antioxidant and anti-inflammatory effects AIM: The purpose of this research is to investigate the role of natural product nano-particles and phytoestrogens (Ziziphus) as an alternative to hormone replacement therapy; each alone or in combination with conventional drugs induced apoptosis in human breast cancer cells in vitro. MATRIAL & METHOD To achieve the hypothesis, we cultured the cells in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum at 37°C in a humidified, CO2 -controlled (5%) incubator. The cells seeded at an appropriate cell density in a 24-well or a six well plate then the cells treated with various treatments. Thereafter, the cells were harvested and used to evaluate the effects of different treatments and explore the possible molecular mechanisms involved through the following techniques. 1- Cell Culture and Viability Assay 2- DNA Fragmentation for Apoptosis Assay 3- Agarose Gel Electrophoresis 4- Gene Expression Analysis Results: The current results indicated that, supplementation of Ziziphus and/or Tannin nanoparticles (NP99) extract able to decrease viability and count of breast cancer (MCF-7) and significantly increased the percentage of DNA fragmentation of the cells. Also, both extracts treatments were able to up regulate the gene expression of apoptotic genes and down regulate the genes of cell growth, so it could be a promising strategy to develop a successful treatment for cancer therapy. Moreover, the combined treatment of both extract with Tamoxifen increased the sensitivity of the drug considering the potential role of phytoestrogens alone and/or in combination in the treatment of breast cancer cells with minimum side effects, which achieves the objectives recommended in control the tumors of the breast. DISSECTION dietary phytochemicals could play an important role in the new drug development for breast cancer and represent a promising option for prevention and/or treatment of this cancer cells. They may exert therapeutic effects by interfering with key cellular events of the tumorigenesis process. In the prevention and therapy of ER+ human breast cancer, the efficacy of the antiestrogen Tamoxifen (TAM) has been well established. However, inherited or acquired hormone resistance reduces TAM activity, and treatment-associated side effects limit the use of high doses of TAM. Therefore, more effective modality is needed to sensitize TAM on ER+/TAM-insensitive breast tumors. Combination treatment is one of the developmental trends for increasing the efficacy and/or minimizing the side effects. From this point of view, the present study found that the Ziziphus extract or Tannin nanoparticles and TAM combination had a synergistic inhibitory effect on the growth of ER+ human breast cancer cells MCF-7 in vitro in part via the synergistic effect on induction of apoptosis. The molecular mechanisms of this synergistic action might be in part via modulation on the expression of the apoptotic suppressor survivin genes, DNA fragmentation percentage and protein content for the most important factors regulating antiproliferation and apoptosis genes as well as proteins are necesary to understand the possible molecular mechanisms by which these agents influence proliferation and apoptotic mechanisms in the breast cancer cells. CONCLUSIONS: - To date, Tamoxifen could not solve the problem of the prevention and treatment of breast cancer fundamentally. - Our finding reveals that Tannin nanoparticles (NP99) extract has the inhibitory effects on breast cancer cells, the molecular mechanisms by which it exerts its activities may partially due to its up-regulation of apoptotic genes expression in both mRNA level and protein level and thus induces cells into apoptosis. - Our results are of significant interest as they highlight the potential usefulness of a particular type of NP99 as an anti-cancer agent. However, these exciting results obviously warrant additional study, including further characterization and development.

Reference:
Khosropanaha MH, Dinavarda A, Nezhadhosseinia A. (2016). Analysis of the Antiproliferative Effects of Curcumin and

**Attachment:**

fig: http://membs.org/membs/uploads/congress_speaker_files/1528353222??? ????????.jpg
Abstract:

In the field of regenerative medicine, bone marrow (BM), umbilical cord blood (UCB) and Wharton's jelly (WJ) have become promising sources of cell therapy. Although various source-derived stem cells have been considered for cell therapy in incurable diseases, there is solid evidence that stem cell populations derived from various sources are different. Multiple previous studies have compared the biology of various stem cell populations from different sources in terms of multiple cellular and molecular aspects. However, determining which stem cell population, as well as the source, is most effective has become a major interest that needs to be explored. In our study, we isolated and compared CD34+, CD133+ and CD271+ cells derived from three different sources including BM, UCB, and WJ in the context of cell morphology, surface markers, differentiation potential as well as immunomodulatory properties. Such information may pave the way for identification and selection of the most suitable cell population & source as a useful model for clinical applications of cell therapy. Results are to be presented at the congress. *These authors contributed equally to this work.
Usher syndrome: from clinic to genetics

Samia, Abdi (1), Makrelouf, Mohamed (2)

(1) saad dahleb university, medecine, CHU Blida, Algeria.
(2) Director of Research Laboratory Biochemistry Genetics, CHU Bab El Oued - Université Alger 1, Algiers, CHU BAB-EL-OUED, Algeria.

Abstract:

Introduction: Usher syndrome is a rare genetic disorder that associated perceptual deafness and retinitis pigmentosa responsible for the onset of blindness. There are 3 types that are differentiated by the affected genes, 5 genes are incriminated in type I, 3 in type II and one in type III. These genes code for a complex of proteins in the eyes and ears, so the symptoms are visual and auditory. Materials and methods: This study was made on a cohort of 10 Algerian families suffering from deafness with ophthalmological signs of retinitis. The phenotypic aspect is studied in collaboration with the ORL team of Blida by an interrogation and a set of complementary examinations such as the audiogram, the PEA, the scanner of the rocks and the brain MRI and the FCP. An ophthalmological examination and a fundus confirmed or disproved the presence of retinitis pigmentosa. The genotypic aspect is studied by looking for the mutations involved by the high-throughput sequencing, this research was done at the Pasteur Institute of Paris after extracting the DNA on blood collected on EDTA tube in the laboratory of genetic biochemistry CHU Bab Eloued. A reconciliation between the clinical data and the genetic data was then performed. Results and discussion: there is a positive correlation between the clinical data and the results of the genetic study. Indeed, the 8 families whose phenotype is that of Usher type 1 syndrome have mutations in the USH1 genes and the other 2 having the phenotype of USH2 have mutations in the USH2 genes. Conclusion: The genetic diagnosis of Usher syndrome is difficult because of the genetic heterogeneity of this syndrome and the length of its genes. but given the total phenotype-genotype correlation observed in this study, it is possible to make early diagnosis of this syndrome by having all deaf children undergo ophthalmological examinations in order to search for clinically delayed Retinis pigmentosa. For this, cooperation between clinicians and biologists is essential or even mandatory.
Systematic screening of antibodies anti HBS, HCV, HIV and-CMV in blood donors at the level of Regional Blood Transfusion Center of Blida University hospital center

Samia, Abdi (1), Ounas, sonia (2)

(1) saad dahleb university, medecine, CHU Blida , Algeria.
(2) Institut de transplantation d'organes et de tissus , Médecine Blida, laboratoire central de biologie, Algeria.

Abstract:

Introduction Majority of viral infections present evocative clinical signs and regress on their own without resorting virological diagnosis by the clinician Viral serology can be used to determine the immune status of an individual against a latent or patent infection and to date an infection. Materials and methods : It is a prospective study conducted on 15932 clinically healthy blood donors and recorded at the regional blood transfusion center of Blida University Hospital. All the collected bloods have undergone a biological examination in this case the viral serology in order to look for possible infections by the HBS, HBC, CMV and HIV viruses. This research was done by the classic ELISA technique which has become the most used technique because it is fast simple specific and adaptable on PLC. Results and discussion : 13 HBS positive cases and 18 HBS positive cases were found No case presents a positive CMV serology. Blida city is not an endemic region for HIV virus,so, no positive HIV case has been found. The discovery of these serological abnormalities compromises the use of these bloods for transfusional purposes. Conclusion: The presence of hepatitis B and C in clinically healthy patients is not always absent from where systematic screening becomes mandatory in blood donors recommendations are needed in the population to fight against viral hepatitis
Nurturing a beast: giving rise to a Doxorubicin resistant triple negative breast cancer cell line

Zureigat, Hadil (1), ALSHAER, WALHAN (2), Souleiman, Mamoun (3)

(1) Student, Faculty of Medicine, University Of Jordan, Jordan.
(2) Research scientist, Cell Therapy Center, The University of Jordan, Jordan.
(3) Student, Faculty of Medicine, University of Jordan, Jordan.

Abstract:

Background: Though much effort is being directed at screening, early detection and treatment of triple negative breast cancer, it remains the most aggressive, usually associated with a very poor clinical prognosis. This cancer not only fails to respond to the traditional targeted chemo-therapy but also demonstrates resistance to other nonspecific agents such as Doxorubicin (Dox). Given that the resistant cells exhibit functional attributes different from those of their parents, we were interested in working with a cell line that demonstrated these cells’ resistance to drugs, specifically Doxorubicin. MDA-MB-231 is a triple negative breast cancer cell line widely utilized in breast cancer research. We set out to transform this cell line into a Dox resistant cell line; in other words, to better understand how to tame this beast, we decided to create it.

Methods: We obtained an MDA-MB-231 cell line and, using an MTT assay, evaluated the anti-proliferative effects of doxorubicin. The data was analyzed and the IC50 of Doxorubicin was established. We then added progressively increasing concentrations of Doxorubicin to the cells over the course of a year. An MTT assay was once again employed to establish the Doxorubicin IC50 of the treated cells. Parental MDA-MB-231 cells served as control.

Results: Our results demonstrated a 14 fold increase in Doxorubicin IC50 in treated cells when compared to Dox IC50 of parental MDA-MB-231 cells (0.537 µM vs 0.0373 µM). This increased value of IC50 reflects the need for a much higher concentration of Doxorubicin to kill the cells when compared to control, reflecting the resistance of these cells to Doxorubicin.

Conclusions: Using increasing concentrations of Doxorubicin, we have successfully established and are currently maintaining a Dox resistant cell line. We now have the liberty of utilizing these cells to develop a more in depth understanding of this cancer. We not only hope to potentiate the effects of currently used drugs but are also interested in understanding the mechanics governing these cells’ resistance. We hope this work will facilitate for others and ourselves means of conquering triple negative breast cancer.
Enhancing the therapeutic efficacy and potency of chemotherapeutics by silencing of genes involved in multidrug resistance mechanism

AL Karaki, Arwa (1), ALSHAER, WALHAN (2)

(1) final msc student, Cell Therapy Center, The University of Jordan, Amman, Jordan. Department of Pharmacology, Faculty of Medicine, The University of Jordan, Amman, Jordan. (2) Research scientist, Cell Therapy Center, The University of Jordan, Jordan.

Abstract:

Enhancing the therapeutic efficacy and potency of chemotherapeutics by silencing of genes involved in multidrug resistance mechanism Arwa Karaki1,2, Dana A. AlQudah1, Suha Wehaibi1, Malek Zihli2, Abdullah S Awidi1, Walhan Alshaer1* 1Cell Therapy Center, The University of Jordan, Amman, Jordan. 2Department of Pharmacology, Faculty of Medicine, The University of Jordan, Amman, Jordan. Abstract Breast cancer is the second leading cause for death worldwide and considered one of the most invasive cancers. Although early diagnosis decreased the mortality of breast cancer significantly, unfortunately most of the patients were responsive to the initial treatment for certain time, latter on patients develop more aggressive tumor forms that were generally resistant to the chemotherapy and radiotherapy. One of the major emerging obstacle for successful therapeutic treatments is Multiple drug resistance; including somatic mutations or epigenetic modifications within drug targets, deregulated apoptosis or survival, adapted signaling pathways or metabolic reprogramming, as well as drug reaching to the targets or drug transport modification within the tumor microenvironment; accordingly chemotherapeutic agents have a limited efficacy in the treatment. so as one of the therapeutic solution in clinical practice is increasing the dose which will result in more adverse side effects. In recent years, more and more attentions have been paid to the gene and chemotherapy drug combination approach. Which has good synergic effect, because gene silencing can inhibit the expression of the target gene, reduces multidrug resistance and accelerates cell death. also, this could reduce the drug dosage, thereby reducing the side effects. Therefore, the combined system of chemotherapeutic drugs and gene therapy is expected to become an effective leading approach for cancer treatment in the coming years for a better curative effect. In our project we will develop a drug resistance cancer cell line, followed by measuring the expression of multidrug resistant related genes. Besides, selecting the candidate genes for silencing by siRNA. The best siRNA by means of therapeutic outcomes will be a candidate for delivery using nanoparticles based delivery system. *Corresponding Dr. Walhan ALSHAER Cell Therapy Center The University of Jordan PO Box: 5825, Amman, Jordan. Office: (+962) 6-5355000 Ext: 23960 Mobile: (+962) 790823678 E-mail: walhan.alshaer@ju.edu.jo

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**Novel Comprehensive Database of Genetic Variants and Phenotypic Associations in Arabs**

Nair, Pratibha (1), Bizzari, Sami (2), El-Hayek, Stephany (3), Al-Ali, Mahmoud Taleb (4)

(1) Senior Researcher, Centre for Arab Genomic Studies, Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences, United Arab Emirates.
(2) Research Assistant, Centre for Arab Genomic Studies, Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences, United Arab Emirates.
(3) , , College de France, France.
(4) Director, Centre for Arab Genomic Studies, Sheikh Hamdan Bin Rashid Al Maktoum Award for Medical Sciences, United Arab Emirates.

**Abstract:**

The high prevalence of genetic disorders in the Arab World contributes to a large proportion of infant mortality and morbidity in the region. The Centre for Arab Genomic Studies has initiated an effort to develop a novel comprehensive open-access database of Arab variants. The new variant database is modeled closely after ClinVar [1] and will host a compendium of genetic disorders, genetic variants, associated phenotypic data, as well as prevalence studies in the Arab population. Data will be culled from bibliographic information extracted from both indexed and non-indexed scientific journals as well as unpublished sources including existing data in other variant databases, diagnostic laboratories, and clinicians. The database will follow the standard regulations and guidelines set by the Human Variome Project for data sharing and presentation; additionally, an ethics committee will incorporate guidelines for the submission and reporting of variants [2]. The goal of this database is to open a window for variant data curation, sharing, and aggregation to the region’s clinicians and researchers, and to have an updated resource pertaining to diseases and genetic variants to benefit physicians, laboratories, patient support groups, and policy makers. In this poster, we detail the extent of information, such as subject data, types of variants, and phenotypic information that will be included and presented in the database. Additionally, the data submission process and advanced user search options will also be discussed.

**Reference:**

Anti-tumor activity of single and combinatorial siRNA through silencing oncogenes involved in pancreatic cancer: Therapeutic approach

Al-Kadash, Abdulfattah (1), Alshaer, Walhan (2)


(2) Research Assistant Professor, Cell Therapy Center, The University of Jordan, Amman, Jordan. Department of Pharmacology, Faculty of Medicine, The University of Jordan, Amman, Jordan, The University of Jordan, Jordan.

Abstract:

Anti-tumor activity of single and combinatorial siRNA through silencing oncogenes involved in pancreatic cancer: Therapeutic approach Abdulfattah Al-Kadash1,2, Dana A. AlQudah1, Suha Wehaibi1, Malek Zihlif2, Abdullah S Awidi1, Walhan Alshaer1* 1Cell Therapy Center, The University of Jordan, Amman, Jordan. 2Department of Pharmacology, Faculty of Medicine, The University of Jordan, Amman, Jordan. Abstract Pancreatic cancer still one of the undruggable tumors despite the vary discoveries about its nature. With its only 5 % five years expected life, metastatic properties, hypoxic surviving signals; delayed diagnosis, poor biomarkers and complex gene expression in the affected tissue make it one of the greatest challenges for medical loftiness. Moreover, with few and unselective chemotherapy available in literature for the treatment of pancreatic cancer with its broad side effects necessitates the findings of new therapeutic approaches. Fortunately, new novel discoveries in the biopharmaceutical and therapeutic field have emerged by recruiting Small interference RNA (siRNA). siRNAs are small double stranded RNAs consisting of 21-23 nucleotides are used now to silence targeted genes through interaction and activation of RNA-induced silencing complex (RISC). Consequently, RISC cleaves only the complementary mRNA expressed by our selected gene. siRNA has been clinically studied and evaluated to silence oncogenes in different cell lines to interrupt the varied biological processes of tumor progression; metastasis, apoptosis, angiogenesis, high replication rate and evading immune system with proved promising results. Having the barbed wire of the pathways activated in pancreatic cancers consolidate the strategy of using the selective properties of siRNA in gene silencing. In this study we are trying to emphasis the role of siRNA in anti-tumor manner in pancreatic cancer as single and combinatorial siRNA by silencing the targeted oncogens and evaluate this effect in the potential for therapeutic approaches. *Corresponding Dr. Walhan ALSHAER Cell Therapy Center The University of Jordan PO Box: 5825, Amman, Jordan. Office: (+962) 6-5355000 Ext: 23960 Mobile: (+962) 790823678 E-mail: walhan.alshaer@ju.edu.jo

Attachment:
Development of Rapid and Efficient AZF Microdeletions Assay by Triplex Real-Time Polymerase Chain Reaction for Male Infertility Diagnosis

Alghadi, Ahmad (1), Yousef, Sana’ (2), Al-Zoubi, Adeeb (3)

(1) Research and Development Officer, Research and Development, Stem Cells Arabia, Jordan.
(2) Chief Research and Development Officer, Research and Development, Stem Cells Arabia, Jordan.
(3) Clinical Assistant Professor of Regenerative Medicine, University of Illinois College of Medicine in Peoria - USA, President, Stem Cells Arabia, Jordan.

Abstract:
The azoospermia factor (AZF) region of the human Y chromosome contains essential genes for spermatogenesis. Microdeletions in the AZF region cause defects in spermatogenesis that may lead to the development of azoospermia and oligozoospermia. Furthermore, microdeletions in AZF region are considered the second most frequent genetic cause for male infertility. To this end, genetic diagnosis for AZF Y chromosome microdeletions has become of great importance in infertility clinics. Several approved methods and commercially available kits are currently available in the market for genetic diagnosis of Y chromosome microdeletions. However, in light of the challenges associated with the currently existing methods as well as increasing demand of this test in infertility clinics, the development of rapid, high throughput and efficient method is now recommended. In this study, we developed, validated and evaluated a rapid, reliable, easy to set up and affordable method based on three-multiplex real-time polymerase chain reaction (RT-PCR) methods for accurate diagnosis of AZF microdeletions with relatively easy result interpretations. The method was developed, validated and implemented under strict compliance with good laboratory practice (GLP) and the guidelines for internal quality control. Our developed method included the most common six AZF regions including sY84, sY86 for AZFa; sY127, sY134 for AZFb; sY254, sY255 for AZFc, in addition to ZFX/ZFY gene and the SRY gene as internal PCR control. We performed a single-center study of diagnostic accuracy to determine the frequency of AZF microdeletions in Jordanian infertile males. At our center, 78 infertile males with azoospermia were screened for AZF microdeletions in the Y chromosome using our developed triplex RT-PCR method. 6 out of 78 infertile males were found to have microdeletions in the AZF region. Among them, four were found to have microdeletion in AZFc, one has microdeletions in AZFa, AZFb and AZFc regions, and one has a microdeletion in the AZFb region. These results were confirmed by external independent laboratories; our results were 100% consistent with the existing methods currently in use by other laboratories. This frequency is comparable to that previously identified in Jordan and other countries. In conclusion, our developed method represents the first effort towards improvement of the molecular diagnosis of the AZF Y chromosome microdeletions. Further multi-center validation with a large sample size to confirm the reproducibility and reliability of our newly developed method for accurate diagnosis is recommended.
The search for factor V Leiden mutation in patients with thrombophilia in the population of Blida

Sonia, Ounnas (1), Samia, Abdi (2)

(1) LABORATOIRE DE BIOLOGIE, Médecine Blida, CHU Blida , Algeria.
(2) saad dahleb university, medecine, CHU Blida , Algeria.

Abstract:
Introduction: Thrombophilia is a multifactorial pathology characterized by a predisposition to potentially serious thromboses that can be complicated by serious pulmonary embolism. The aim of this work is to study the different biological anomalies at the origin of this disease, in particular the Leiden factor V mutation. Patients and methods This is a retrospective descriptive study over 5 years that included 96 patients consulting for thrombosis at the cardiology and internal medicine department of the Frantz Fanon Teaching Hospital in Blida, Algeria. The age of this population is between 20 and 83 years. The search for factor V Leiden mutation was made by the RPCA test or activated protein C resistance. Results 96% of patients have venous thrombosis and 3% have arterial thrombosis. Among this patient population, 9.4% have a positive RPCA test, a mutation in the factor V Leiden of coagulation. Conclusion The results found are different from the international data that report higher figures in the Caucasian and Asian population and lower in the Middle East population. A genetic study looking for this mutation in the at-risk population is desirable. Keywords Thrombophilia, vascular thromboses, V Leiden factor.
Genotype detection of fimH gene of Acinetobacter baumannii isolated from different clinical cases.

AlShwaikh, Rana (1)

(1) professor , University of Baghdad , Department of Biology, College of Education , , University of Baghdad, Iraq.

Abstract:

Forty isolates were obtained of Acinetobacter baumannii from (200) isolate, the isolates were collected from different cases including :- wounds, burns, Stool , Urinary tract infection urine, Respiratory tract infection and blood sample. for the period between 1/9/2016 to 30/11/2016 which included 50 isolates from blood, 20 isolates from urinary tract infections, 30 isolates from wound infections, 40 isolates from burn infections and 25 isolates from stool samples from several hospitals in the of Baghdad city (Central Children's Hospital, Al Karama Hospital, Karkh General Hospital, Al-Ameen Medical City Hospital, Educational Labs, Baghdad Teaching Hospital, Child Protection Hospital, Burns and Wounds Hospital). After identification 40 isolates confirmed to be Acinetobacter baumannii included 9 isolates from blood, 1 isolate from urinary tract infections, 4 isolates from wound infections, 8 isolates from burns and 14 isolates from stool sample. Genotypic detection for some virulence genes of Acinetobacter baumannii which included fimH, the result revealed that the fimH gene was present in (19) isolates (47.5%) of Acinetobacter baumannii. The result showed (7 isolate (17.5%) of Stool and blood has fimH gene to each of them and Burns (3) isolates (7.5%) has fimH gene and ( 2 ) isolate ( 5% ) of respiratory tract infection has fimH gene and finally only (1) isolate (2.5 %) of Wound infection. While all isolated for each of the urinary tract infections doesn't the fimH gene. The gel electrophoresis showed that the molecular weight of fimH gene was 508 bp. Sequential analysis detection of fimH showed three silent mutations that did not affect the amino acid translation.
Resistance of Swimmer and Swarmer cells of Proteus mirabilis to antibiotic

abdalhadee, Luma (1)

(1) Assistant Professor, Biology Department, Baghdad, Iraq.

Abstract:

Twenty one isolates of Proteus bacteria were collected from different clinical and animals samples for the period from October 2017 to November 2017. All isolates were identification depending on microscopic characterization, biochemical tests and Vitek 2E compact system, it appears that eighteen isolates were belong to P.mirabilis. This study was aimed to antibiotics resistance (Penicillin, Tetracycline, Nitrofurantoin and Cefoxitin) on Proteus mirabilis swimming and swarming motility. All P. mirabilis isolates were capable of swimming and swarming over semi-solid media. The results show that the resistance of swimmer cells and swarmer cells to antibiotics is variable depending on the source of isolate and the type of method and swarmer cells more resistance to antibiotic than swimmer cells.
Mesenchymal stem cells labelling using magnetic particles for in vitro applications

AlKharji , Reem (1)

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Abstract:
Cell-based therapy is not a new concept; it is considered as one of the most promising approaches for treating diseases and for regenerative medicine. In addition, effective cell therapy can greatly benefit from the ability to monitor transplanted stem cells post-intervention. Mesenchymal stem cells (MSCs) represent one of the leading candidate populations for regenerative medicine. These cells, which are present in adult tissues, are non-hematopoietic stem cells with multipotent capacity toward a range of mesodermal lineages. Superparamagnetic iron oxide nanoparticles (SPIONs) represent contrast agents offering a possible way to track labelled cells after administration using MRI. Moreover, we have demonstrated that these magnetic particles (MPs) do not affect cell viability, proliferation, differentiation or migration. The aim of the present study was to determine the ability to use these iron particles to label mesenchymal stem cells (MSCs) and test their potential to control cell migration when exposed to a magnet. This aim was achieved by culturing labelled and unlabelled cells in 2D and 3D models, in presence or absence of magnet. Significant response to magnet exposure was observed in 2D culture where 76% of labelled cells moved to the magnet side when compared to unlabelled cells. There was only 45% of unlabelled cells found to have moved to the magnet side. In addition, 64% of labelled cells moved to the magnet side in a 3D culture model, while the unlabelled cells showed around 50% cells moving to the magnet side. In summary, we have shown that MSCs can be labelled with MPs in vitro, and this strategy can contribute to improving the spatial tracking of transplanted stem cell, and therefore improve their efficiency for therapeutic applications.

Reference:
Cytokine mediated effects of human Wharton’s Jelly stem cell (hWJSC) extracts in inhibition of an ovarian cancer cell line (OVCAR3) in vitro

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Abstract:

Introduction: Malignant ovarian cancers are one of the most lethal gynaecological cancers and the fifth common causes of death in women. Conventional therapy are effective in at least 70% of early diagnosed ovarian cancers, but the emergence of multi-drug resistance leads to cancer relapse within a short span of 1 - 2 years [1]. Early diagnosis usually has better prognosis while in advanced cases despite best possible treatment the mortality rate is high and the 5 year survival rate is approximately 30% [2]. Human Wharton’s jelly stem cells (hWJSCs) has been reported to inhibit various human cancers both in in vitro and in vivo animal studies [3-6].The precise inhibitory effects of hWJSCs mediated inhibition is not known. Tumours in general represent chronic inflammation and cytokines are known to exert pleiotropic effects which can contribute either to tumour progression or inhibition. As such in the present study we evaluated the cytokine secretion profile in vitro following treatment of OVCAR3 with hWJSC extracts namely its conditioned medium (hWJSC-CM) and cell lysate (hWJSC-CL). Materials and Methods: Ethical approval was obtained to derive hWJSCs from human umbilical cords. OVCAR3 was purchased from ATCC. The effects of hWJSC extracts (hWJSC-CM and hWJSC-CL) was tested on OVCAR3 for the secretory cytokine profile using cell culture supernatant of OVCAR3 collected at 48 h following treatment of OVCAR3 cells with hWJSC-CM (50%), hWJSC-CL (15µg/ml) and paclitaxel (2.5nm, 5nm, 10nm, 20nm and 30nm). Cytokine analysis was performed using multiplex immune-bead assay kit for human cytokines 30 plex panel. The assay was performed according to the manufacturer’s instructions, in a 96-well plate format and the results analyzed using MAGPIX® instrument (Luminex, USA). Data obtained was analyzed using the Luminex® xPONENT® multiplex assay analysis software. Results: The derived hWJSCs fulfilled the minimal criteria for MSCs such as having spindle shaped fibroblastic morphology, plastic adherence and expression of MSCs related CD markers (Positive expression for CD29, CD44, CD73, CD90, CD105 and negative expression for CD34 and CD45). Cytokines analysis from the cell culture supernatant of OVCAR3 following treatment with hWJSC-CM (50%), hWJSC-CL (15µg/ml) and paclitaxel (2.5nm, 5nm, 10nm, 20nm and 30nm) for 48 h demonstrated either increases or decreases in the secreted cytokine profile in the treatment groups compared to the control (Figure 1A-D). The cytokine IL-1b demonstrated decreases with both hWJSC-CM and hWJSC-CL; IL-1RA showed mild increases with hWJSC-CM and hWJSC-CL and IL-2 did not show much changes with hWJSC extracts (Figure 1A). The cytokines IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13 demonstrated decreases with hWJSC-CM and hWJSC-CL compared to the control. Paclitaxel demonstrated mostly a decrease for all above cytokines at higher concentrations of 5nm and 10nm (Figure 1B). The cytokines IL-15, IL-17 as well as the growth factors G-CSF, b-FGF, EGF and VEGF demonstrated decreases with hWJSC-CM and hWJSC-CL compared to the control. HGF demonstrated decrease with hWJSC-CL alone. The cytokines IFN-a, IFN-g, TNF-a and the chemokine IP-10 demonstrated decreases with both hWJSC-CM and hWJSC-CL compared to the control (Figure 1C). The chemokine MIP-1a, MIP-1b, RANTES, MCP-1 and MIG demonstrated decreases with both hWJSC-CM and hWJSC-CL, while there was no changes in EOTAXIN compared to the control (Figure 1C). Paclitaxel demonstrated mostly a dose dependent decrease for all above cytokines and growth factors except for VEGF which showed no changes (Figure 1D). Discussions and Conclusions: Both hWJSC-CM and hWJSC-CL led to inhibition of OVCAR3 cells by either increasing/decreasing of the cytokines, chemokines, growth factors and ensuing interplay between them. The cytokine secretory pattern provided the possible mechanism of OVCAR3 cell death also confirmed with other in vitro study findings such as altered cell morphology, cell cycle arrest, induction of apoptosis, decreasing the expression of CSC markers and genes regulation (data not shown). The cytokine expression pattern may vary according to the tumour type thus contributing as biomarkers which help in both diagnosis and prognosis [7]. Tumours usually escape immune surveillance; however, the cytokinases that prevail in the tumour
microenvironment may predispose them to events leading to their apoptosis and tumour control [8]. IL-2 activates natural killer cells and thus have anti-tumour properties. TNF-a predisposes to apoptosis of cancer cells via activation of caspases [9]. IL-6, IL-8, IL-10, RANTES, MCP1 have been earlier reported to be increased in malignant ovarian cancer [10]. IP-10, IL-10 and MCP-1 are expressed at higher levels in ovarian cancer and promote tumour growth and metastasis in an autocrine manner [11]. The growth factors bFGF, EGF, HGF and VEGF are implicated in tumour cell proliferation, growth and differentiation and their treatment is reported to increase the level of telomerase activity in ovarian cancer cell lines [12], which clearly indicate the role of these growth factors in tumour survival and progression. Interestingly, our study has identified that most of the above cytokines/chemokines were decreased following treatment with hWJSC extracts and therefore we conclude that cytokine therapy or their regulation may be useful in ovarian cancer inhibition.

Reference:


Attachment:
Gauthaman et al - MEMBS - Abstract 2 - Figure 1A-D: http://membs.org/membs/uploads/congress_speaker_files/1529084477Gauthaman et al - MEMBS - Abstract 2 - Figure 1A-D.tif
Resveratrol-mediated modulation of intracellular ROS levels elicits a biphasic effect on PKC activity and endothelial cells survival

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Abstract:

Reactive oxygen species (ROS)-induced cellular damage is associated with many pathological conditions (Forrester et al., 2018). In this context, (ECs) plays a pivotal role in maintaining the cardiovascular homeostasis, and ROS-induced ECs dysfunction is a critical step in the onset and progression of many cardiovascular diseases (CVD) including atherosclerosis and hypertension (Incalza et al., 2018). Dietary intake of natural antioxidant (NA) is thought to provide cardiovascular benefits (Bielli et al., 2015). Nevertheless, many in vivo studies and clinical trials have failed to demonstrate the postulated cardio-protection of NA. Resveratrol (RES) is a natural polyphenolic antioxidant present in the diet (Liu et al., 2018). Although many scientific evidences show that RES decreases oxidative stress, we have previously demonstrated that high levels of RES can exert, paradoxically, a pro-oxidant effect eventually leading to cell death. In this regard, demonstrated that high dosage of resveratrol induces a mitochondria-dependent pro-oxidant damage of human ECs, which is mediated by cytochrome CYP2C9 ROS production and AKT downregulation (Pasciu et al., 2010; Posadino et al., 2013; Posadino et al., 2015) Here we further deepen the study of the molecular mechanism of resveratrol-induced EC-damage showing that high doses of resveratrol negatively impact Protein Kinase C (PKC) activity inducing ECs apoptotic death as evidenced by the increased DNA fragmentation. Downregulation of PKC activity correlated with the inhibition of Bcl-2 gene expression and the increase of Bax gene expression, which play respectively an anti- and pro-apoptotic role in cell fate. Ulterior molecular analyses indicated the inhibition of c-myc and ODC gene expression, two genes involved in the regulation of cell cycle progression. Indeed, impairment of cell cycle progression was further confirmed by Cyclin D1 protein downregulation and accumulation of ECs in S or G1/G0 phase depending on the used resveratrol dosage Beside provide new molecular insight concerning the impact of NA on ECs, our results also suggest that identification of an optimal dosage is essential to have a great bene?it–risk ratio in all the forms of resveratrol consumption.

Reference:
