3RD ANNUAL AUB BIOMEDICAL RESEARCH DAY

THEME: BRIDGING BASIC AND TRANSLATIONAL RESEARCH

Charles W. Hostler Student Center Auditorium

Saturday, February 16, 2013

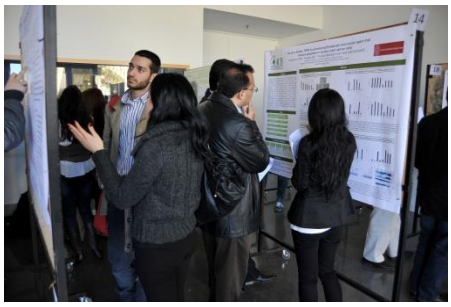
8:30 am - 2:00 pm

Table of Content

Awardees of the 2012 AUB Basic Biomedical Research Day

- **Patricia Moghames**, FAFS: *H.pylori infection in Lebanon: Prevalence, risk factors and association with metabolic syndrome*
- **Zeina Dassouki**, FM: *Both PML nuclear bodies and RNF4 are required for arsenic/interferon-induced degradation of the HTLV-I oncoprotein Tax*
- **Kawthar Braysh**, FM: *Role of mTOR in Podocyte Injury and albuminuria in Type 1 Diabetes*
- **Hayat Harati**, FM: *Galactosylceramide (GalCer) as potential treatment for juvenile neuronal ceroid lipofuscinosis (JN*CL)






Schedule of events

8:30 am - 9:00 am	Welcome note **Dr. Ayad Jaffa**, Assistant Dean for Interdisciplinary Programs Program Projects in Biomedical Research and Innovative Biomedical Research Grants Award Presentation *Provost Ahmad Dallal, Dean Mohamed H. Sayegh* 2013 Farouk Jabre Award Presentation *Provost Dallal, Dean Sayegh, Trustee Jabre*
9:00 am - 10:00 am	Key note speaker to be introduced by Dr. Samia Khoury, Associate Dean for Translational and Clinical Research **Dr. David Bickers,** Chair of the Medicine and Health Committee of the AUB Board of Trustees, and Professor and Chair of the Department of Dermatology at Columbia University Medical Center ***Title**: Precision Medicine: Mechanism Driven Targeted Therapy of Non-Melanoma Skin Cancer*
10:00 am - 10:30 am	Coffee break <u>Presentations by the 2012 Farouk Jabre Award recipients</u>
10:30 am – 10:50 am	**Dr. Georges Nemer**, FM and **Dr. Zakaria Kambris**, FAS: *The nephrin gene: A renal gene implicated in cardiovascular diseases*
10:50 am - 11:10 am	**Dr. Marwan Sabban**, FM and **Dr. Hala Muhtasib**, FAS: *Targeted therapy of breast cancer stem cells and metastasis by the hypoxiaactivated quinoxaline 1,4-dioxide DCQ*
11:10 am - 11:40 am	**Dr. Samira Kaissi,** Managing Director, Basic Science Research**:** *What makes a good research place a great research place? And other perplexing questions…*
11:40 am - 2:00 pm	Poster viewing followed by lunch, award presentation for the top 4 posters and closing

Objectives

- serve as a platform to bring together the research community of different AUB faculties and to showcase the biomedical research performed at AUB
- provide an intellectual environment for scientific exchange among the various researchers at AUB
- provide a platform for students, postdoctoral fellows and junior investigators to present their scientific findings and to foster collaboration within the AUB family of investigators

Eligibility

- Students
- Trainees
- Residents
- Research Assistants
- Fellows
- Post docs

Organizing Committee

Chairperson:

Ayad Jaffa, Assistant Dean of Interdisciplinary Programs, FM, Department of Biochemistry and Molecular Genetics

Members:

- Hala Muhtasib, FAS, Department of Biology
- Kamal Bouhadir, FAS, Department of Chemistry
- Marwan Sabban, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Nahla Hwalla, FAFS, Dean
- Fadi Karameh, FEA, Department of Electrical and Computer Engineering
- Ghassan Dbaibo, FM, Department of Pediatrics and Adolescent Medicine and Department of Biochemistry and Molecular Genetics
- Zaher Dawy, FEA, Department of Electrical and Computer Engineering
- Nadine Darwiche, FM, Department of Biochemistry and Molecular Genetics
- Assaad Eid, FM, Department of Anatomy, Cell Biology and Physiological Sciences
- Soha Yazbek, FHS, Medical Laboratory Sciences Program
- Nada Melhem, FHS, Medical Laboratory Sciences Program
- Yumna Maalouf, FM, Medical Dean's Office
- Khawla Malla, FM, Interdisciplinary Academic Programs

Keynote Speaker

David R. Bickers, M.D.

- Carl Truman Nelson Professor and Chair, Department of Dermatology,

  College of Physicians and Surgeons, Columbia University Medical Center, NY
- Vice-Chair for Medicine and Health of the Board of Trustees of the American University of Beirut, Lebanon

Dr. David R. Bickers has been the Carl Truman Nelson Professor and Chair of the department of Dermatology at the College of Physicians & Surgeons of Columbia University Medical Center in New York since 1994. He received an A.B. degree in Classics (Pre-Medical) from Georgetown University in 1963 and the M.D. degree from the University Of Virginia School Of Medicine in 1967. After an internship in Internal Medicine at the University of Iowa Hospitals, and two years in the United States Air Force, he was a resident in Dermatology at the Skin and Cancer Unit of the New York University Medical Center beginning in 1970. Thereafter he completed a NIH-supported research fellowship at the Rockefeller University in New York City working with the late Professor Alvito Alvares in the laboratory of Professor Attallah Kappas. During that time he was an RJ Reynolds Scholar in Clinical Medicine and was also a faculty member in the department of Dermatology at Columbia until 1977 when he became Chair of the department of Dermatology at Case Western Reserve University.

Dr. Bickers is a past president of the Dermatology Foundation and the American Board of Dermatology, a past chair of the General Medicine “A” Study Section of the National Institutes of Health and a past Secretary-Treasurer and President of the Society for Investigative Dermatology. He is a past-president of the Medical Board of the New York Presbyterian Hospital. He is a member of the American Society for Clinical Investigation and the Association of American Physicians. He is an Honorary Member of the American Academy of Dermatology, the German Dermatological Society, the Austrian Dermatological Society and the Society for Investigative Dermatology. He is Vice-Chair for Medicine and Health of the Board of Trustees of the American University of Beirut in Lebanon.

2012 Farouk Jabre Award Recipients

- **Dr. Georges Nemer**, FM and **Dr. Zakaria Kambris**, FAS: *The nephrin gene: A renal gene implicated in cardiovascular diseases*
- **Dr. Marwan Sabban**, FM and **Dr. Hala Muhtasib**, FAS: *Targeted therapy of breast cancer stem cells and metastasis by the hypoxiaactivated quinoxaline 1,4-dioxide DCQ*

ABSTRACT #1

MI-RNA-196C* EXPRESSION IN FOLATE-INDUCED ADULT MAMMALIAN CNS REGENERATION AFTER INJURY

Dany M.[1,2], Haidar A.[1,2], Jackson A.[2], Hariharan N.[2], Meethal SV.[2], Iskandar BJ.*[2]

[1]Faculty of Medicine, American University of Beirut, Lebanon;
[2] Department of Neurological Surgery, University of Wisconsin- School of Medicine and Public Health, Madison, WI.
** Address correspondence to Dr Iskandar, Department of Neurological Surgery, University of Wisconsin, 600 Highland Avenue, K4/832, Madison, WI 53792.*
**E-mail: iskandar@neurosurgery.wisc.edu*

Introduction
It is well established that CNS neurons of mammalian adults are incapable of regenerating axons after injury. Folic acid supplementation has been found to induce regeneration and repair of the adult CNS in rodents with spinal cord injury. Further studies showed that folic acid turned out to be a regulator of the expression of genes related to neuronal regeneration through the epigenetic mechanism of DNA methylation in a biphasic and dose-dependent manner. Recent evidence suggests that folate and DNA methylation modulate the expression of microRNAs, a new class of small non-coding RNAs that regulate gene expression. Microarray data from our laboratory has shown that the expression of several miRNAs including miRNA-196c* is altered after injury but then restored to normal with folate supplementation. Accordingly, we we attempt in this study to validate the microarray results using RT-PCR.

Methods
Three groups of adult male Sprague-Dawley rats were used: Group A- control with no injury and double distilled water (DDW) treatment; Group B- sharp surgical injury to the cervical spinal cord with DDW treatment; and Group C- sharp surgical injury to the cervical spinal cord with folate treatment (40, 80, 160, 400, or 800 µg/Kg). After two weeks, tissues from the spinal cords were homogenized and total miRNA was extracted. The cDNAs formed from the miRNAs underwent Real Time PCR with a primer specific to miRNA-196c* for the determination of its expresison levels.

Results
We show that miRNA-196c* expression levels are significantly lower in samples from injured vs. uninjured spinal cords. In injured animals pretreated with folic acid at all the doses tested, the original expression levels of miRNA-196c* are restored and significantly increased compared to untreated animals.

Conclusion
miRNA expression plays a role in folate-induced regeneration mechanisms, likely thorugh an interplay between miRNA and DNA methylation. Our findings may offer new insights into therapeutic approaches to CNS injury via modulation of miRNA expression.

Keywords: folic acid, CNS regeneration, miRNA

ABSTRACT #2

CDK2 REPRESSION IS NECESSARY FOR CELLULAR SENESCENCE AND OCCURS IN A P-53-DEPENDENT MANNER

Hasan Zalzali PhD, Bilal Nasr MS, Mohamad Harajly MS, Raya Saab MD

Department of Pediatric and Adolescent Medicine, AUBMC
Corresponding author: Hasan Zalzali, post-doctoral fellow.
*Email: hz13@aub.edu.lb

Introduction
Cellular senescence is a tumor suppressor mechanism that occurs in response to multiple tumor promoting insults, such as oncogenic stress, DNA damage, telomere shortening, and others.

Methods
Using mouse and human fibroblast cell lines, and an in-vivo model of Cyclin D1-driven brain tumor, we investigated the role of Cdk2 in cellular senescence.
Results: p53 activation resulted in transcriptional repression of Cdk2 during the induction of p53-dependent senescence, both in vitro and in vivo. Ectopic CDK2 expression was sufficient to bypass senescence after oncogenic insult in vitro, uncovering a central role of CDK2 repression in p53-dependent senescence. CDK2 inhibition, either pharmacologically or by use of a dominant-negative CDK2 transcript, was sufficient to block proliferation and induce early senescence after oncogenic insult in vitro. In contrast, CDK2 repression in unstressed mouse and human fibroblasts resulted in cell cycle block without features of senescence, suggesting the need for other effectors of p53 for the senescence phenotype. Pharmacologic inhibition of CDK2 in vivo, using a Cyclin D1-driven brain tumor model of cellular senescence, was successful in decreasing proliferation and promoting early cellular senescence in p53-wild type cells. CDK2 inhibition in p53-null animals resulted in some decrease in cell proliferation, but was not sufficient to induce senescence.

Conclusion
CDK2 repression during cellular senescence occurs at a transcriptional level in a p53-dependent manner, and is necessary for p53-dependent senescence induction. However, it is not sufficient in the absence of p53 activity. These results may explain the weak efficacy of CDK2-inhibitors in cancer therapy, specifically in tumors where p53 is nonfunctional. We propose that CDK2 inhibition may be used in cancer prevention prior to p53 loss, as in premalignant lesions. Ongoing work is aimed at identifying cooperating effectors of p53-induced senescence, to investigate pathways that can be inhibited in parallel with CDK2, for optimal tumor response.

Keywords: cellular senescence, CDK2, tumor suppression

ABSTRACT #3

EPIGENETIC THERAPY IN RHABDOMYOSARCOMA

O. Sarkis, R. Saab
Department of Pediatric and Adolescent Medicine, AUBMC
Corresponding author: Omar Sarkis, Masters Student.
*Email: oss05@aub.edu.lb

Introduction
Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children with two subtypes: embryonal rhabdomyosarcoma (ERMS) and alveolar rhabdomyosarcoma (ARMS). With current treatment strategies, most children with recurrent or high-risk disease fare poorly, identifying a need for novel therapeutic approaches. We are investigating the therapeutic potential of the epigenetic modulating drugs Valproic Acid (VPA) and SAHA, both of which function as histone deacetylase inhibitors (HDACi).

Methods
We performed cell accumulation and cell cycle analysis studies on a panel of RMS cell lines, using both both embryonal (Rh18, Rh36, JR1) and alveolar (Rh30, Rh41, Rh28) cell lines.

Results
Cell accumulation assessed by MTT assay showed that VPA had some effect on cell proliferation at high doses, but that SAHA effectively inhibited cell proliferation in all cell lines tested, at concentrations as low as 1uM. This inhibition of cell accumulation was persistent even days after drug withdrawal, suggesting irreversible cell cycle exit. Cell cycle analysis showed that SAHA led to accumulation of RMS cells in the G1 phase after 2 days of treatment. In addition, most treated cells assumed a spindle-shaped and elongated pattern, suggestive of enhanced differentiation.

Conclusion
This data identifies SAHA as an interesting possible therapeutic agent in RMS, likely acting via epigenetic reprogramming of RMS cells. We are currently investigating the mechanistic details of the cell cycle inhibitory activity of SAHA in RMS cells, and possible effects on cellular differentiation and senescence.

Keywords: rhabdomyosarcoma, valproic acid, SAHA, epigenetic therapy

ABSTRACT #4

H3K9M2E3-BASED CHIP-SEQ ANALYSIS UNVEILS GLOBAL FUNCTIONAL RE-PROGRAMMING IN CYCLIN D1-DRIVEN CELLULAR SENESCENCE

Mohamad Harajly[1], Noel Ghanem[2], Raya Saab[1]

[1]Department of Pediatric and Adolescent Medicine, AUBMC
[2]Department of Biology, AUB
Corresponding author: Mohamad Harajly, Research Assistant.
*Email: mh135@aub.edu.lb

Introduction
Senescence is a permanent G1 cell-cycle arrest that can be triggered by acute stresses such as activated oncogenes and chemotherapeutic agents, and serves as a barrier to cancer development. Senescent cells exhibit distinct chromatin features compared with proliferating cells, known as senescence-associated heterochromatin foci (SAHF). These foci are enriched with histone H3 trimethylated at lysine 9 (H3K9me3), and are thought to impose a repressive state on regional genes.

Methods
We performed sequencing of elements associated with H3K9me3 by ChIP-seq technology, using an in vivo model of senescent pineal cells driven by Cyclin D1 oncogenic signaling, and compared them to non-senescent transformed (Cyclin D1 expressing but p53-null) counterparts.

Results
We identified a set of genes whose H3K9me3 enrichment level was significantly changed (>2-fold). We also analyzed gene expression profiles on Affymetrix transcriptome chips. We found that about 50% of genes associated with H3K9me3 exhibited significant reduction in expression, while other genes were either upregulated or shown no change in expression. Gene ontology analysis clustered the genes into several functional groups, including cell-cycle control, transcription regulation, protein metabolism, cellular differentiation, and cellular adhesion. Notably, the gene ontology clustering showed that genes associated with H3K9me3 in senescent cells clustered into distinctly different functional programs than those associated with H3K9me3 in cells that had bypassed senescence.

Conclusion
We are currently conducting validation studies on separate sets of samples, and further validating hits by real-time PCR analyses. In essence, our data indicate that genome-wide changes of senescence-associated H3K9me3 are informative regarding a functionally profoundly re-programmed phenotype of the senescent condition beyond a mere cell-cycle arrest.

Keywords: cellular senescence, heterochromatin, Cyclin D1, tumor suppression

ABSTRACT #5

EFFECTS OF HIGH FREQUENCY OSCILLATORY VENTILLATION (HFOV) PRESSURE SETTINGS ON OXYGENATION AND VENTILATION OUTCOMES IN VERY LOW BIRTH WEIGHT (VLBW) PRETERM INFANTS

Nathalie E. Chalhoub, Rachana Singh, Sherry E. Courtney, and Robert H. Habib

Department of Internal Medicine, American University of Beirut, Beirut, Lebanon
Division of Newborn Medicine, Baystate Children's Hospital, Springfield, MA, USA
Division of Neonatology, Arkansas Children's Hospital, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA
*Email addresses:
Nathalie E. Chalhoub: nc13@aub.edu.lb
Robert H. Habib: rh106@aub.edu.lb
Presenter: Nathalie E. Chalhoub, postdoctoral fellow/ research assistant

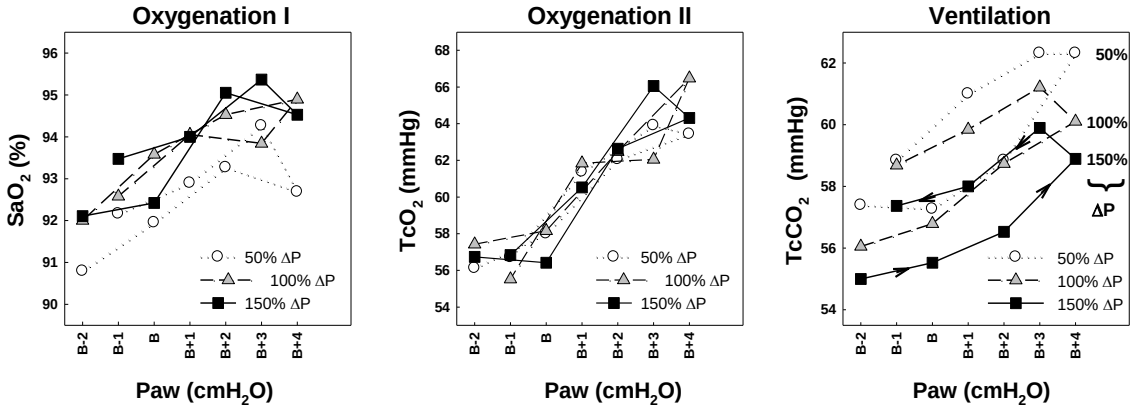
Background
Effects of varying the HFOV mean airway (Paw) and oscillatory amplitude (ΔP) pressures on gas exchange in VLBW infants ventilated for respiratory distress is incompletely elucidated. We hypothesized that changes in Paw will mainly affect oxygenation status while the effects of ΔP will be limited to ventilation.

Aims
To determine the effects of systematically varying Paw and ΔP on lung oxygenation and ventilation in HFOV-supported infants.

Methods
We collected oxygen saturation (SaO_2) and transcutaneous gas exchange ($TcCO_2$ and TcO_2) data in 19 VLBW infants [8/11 M/F; 25.7±1.38 wk (gestation), 767±205 g (birth weight), 6.3±7.9days (age), FiO_2 (0.21-0.5)] at multiple Paw and ΔP varied relative to the baseline (B) clinical values (8.5±2.2and 18.1± 6.5cmH_2O, respectively). Measurements included a stepwise increase in Paw (**Inflation:** B-2, B, B+2, B+4) followed by 3 decreasing settings (**Deflation:** B+3, B+1 and B-1). At each Paw, measurements were repeated for 3 ΔP levels (100% baseline, then ±50% in random order).

Results
SaO_2 and TcO_2 were improved with increasing Paw, yet oxygenation values did not exhibit significant Inflation-Deflation hysteresis.[Fig(Left, Middle)] Both SaO_2 and TcO_2 were unchanged with ΔP despite relatively large changes (±50%). $TcCO_2$ showed a predominant increase with increasing Paw at all ΔP, and with notable Inflation-Deflation hysteresis. [Fig (Right)] TcCO2 was, however, reduced significantly and systematically as ΔP increased for all Paw.

Conclusions
In VLBW infants supported with HFOV, oxygenation is primarily determined by the functional lung volume which is defined by Paw. Ventilation is improved (lower$TcCO_2$) by increasing ΔP, but -on average- $TcCO_2$ was also increased at higher Paw. The latter may be explained by the nonlinear quasi-static lung P-V curve characteristics such that lower effective compliance at high Paw reduces oscillatory tidal volumes (ventilation) and in turn increases$TcCO_2$.



Keywords: HFOV; mean airway pressure; oscillatory amplitude; oxygenation; ventilation

ABSTRACT #6

WILD AND CULTIVATED ACHILLEA FALCATA: A RICH SOURCE OF SESQUITERPENE LACTONES WITH ANTICANCER PROPERTIES

Rita Tohme[1, 2, 3], Lamis El-Aaraj[2, 3], Hala Muhtasib[1, 2], Salma N. Talhouk[2, 4], Tarek Ghaddar[2, 3], Najat A. Saliba[2, 3] and Nadine Darwiche[2, 5, *]

Department of Biology[1], AUB Nature Conservation Center[2], Departments of Chemistry[3], Landscape Design and Ecosystem Management[4], and Biochemistry and Molecular Genetics[5], American University of Beirut, Lebanon
*Corresponding Author: nd03@aub.edu.lb
Rita Tohme is a Master's student at the Biology Department.

Background and Aims
Cultivating medicinal plant species is an adequate strategy to meet the growing demand for a continuous supply. *Achillea falcata*, a plant endemic to the Middle Eastern region, is one of the most commonly used plants in Lebanese folk medicine. The main sources of bioactivity in this plant are the sesquiterpene lactones, which exhibit a high and selective potency against numerous cancer cells while sparing normal ones. The general aim of our project is to compare the anticancer properties and sesquiterpene lactones profile from wild *versus* cultivated species in order to promote the sustainable use of *Achillea falcata*.

Methods
The plant was successfully cultivated at the Agriculture Research and Education Center facility of AUB. The aerial parts of both cultivated and wild *Achillea falcata* were fractionated using an acid-base extraction procedure. Plant extracts rich in sesquiterpene lactones were tested for their anti-proliferative properties against HCT-116 colorectal human cancer cell line. The concentrations that decrease the growth of cancer cells were determined using an MTT proliferation assay. The extracts were further fractionated using liquid chromatography. The collected sub-fractions were tested for their anti-proliferative properties and were further purified using solid phase extraction. Three novel sesquiterpene lactones were identified *via* several spectroscopic methods. Cell proliferation and half maximal inhibitory concentrations (IC_{50}) were assayed against HCT-116 after 24 hours of treatment up to 100 µg/ml concentrations. The cytotoxicity of the three sesquiterpene lactones was also determined at 6 hours treatment up to 100 µg/ml by lactate dehydrogenase release.

Results
We found that wild and cultivated plant species have similar sesquiterpene lactones profile although the IC_{50} of extracts rich in sesquiterpene lactones were approximately 40-50 % lower for wild *Achillea falcata* compared to the cultivated plant. The calculated IC_{50} values for the three isolated novel sesquiterpene lactones ranged between 5 and 15 µg/ml. Interestingly, these sesquiterpene lactones were not cytotoxic at 6 hours treatment up to 100 µg/ml.

Conclusion
Cultivated *Achillea falcata* can serve as a rich source of sesquiterpene lactones with anticancer properties, which will help the conservation of this medicinal plant.

Keywords: Medicinal plants, sesquiterpene lactones, anticancer.

ABSTRACT #7

SUCCESS OF ENDOSCOPIC LASER CYCLOPHOCOAGULATION AFTER PRIOR TRANSSCLERAL CYCLOABATION

Christiane Al-Haddad, MD, Anita Barikian, MD, Baha' Noureddin, MD, Ziad F. Bashshur, MD

Department of Ophthalmology, American University of Beirut
Corresponding Author: Ziad F. Bashshur, MD
*Email: zb00@aub.edu.lb
Anita Barikian, MD: Research fellow

Background and Aim
Cyclophotocoagulation lowers intraocular pressure (IOP) by ablating the ciliary processes; this is traditionally performed using a transscleral approach. Endoscopic cyclophotocoagulation (ECP) treats ciliary processes using the endoscope under direct visualization as opposed to the earlier method of transscleral cyclophotocoagulation (TCP). Our aim is to study the efficacy of ECP in eyes with persistent glaucoma despite prior treatments with TCP.

Methods
This is a retrospective chart review of glaucoma patients at the American University of Beirut Medical Center over one year who underwent ECP after failing TCP treatments. Success was defined as postoperative IOP≤21 mmHg, with or without medications and without procedure related complications.

Results
Eleven eyes of 10 patients were included: 3 females and 7 males. Mean age was 40±23.2 years at time of treatment. Glaucoma diagnoses included: pseudophakic glaucoma(2), congenital glaucoma (3), aphakic glaucoma (2), chronic angle closure glaucoma (2), and primary open angle glaucoma (3). Mean number of prior ocular surgeries (including glaucoma surgeries) was 2.2±1.4 and mean number of prior TCP was 1.5±1.0. Mean arc of treatment of ECP was 297±90 degrees. IOP decreased significantly by 60% from a preoperative mean of 34±10.4 mmHg to a postoperative mean of 13.3±4.9 mmHg ($P<0.001$), with an absolute reduction of 21 mmHg over a mean follow-up of 8.2±4.1 months. The number of anti-glaucoma medications decreased significantly from 3.8±1.1 preoperatively to 1.5±1.0 postoperatively, ($P<0.001$). No complications were encountered. 100 percent of eyes achieved IOP below 21 mmHg.

Conclusion
Ciliary processes may be missed within the treatment zone of TCP since the procedure is not done under direct visualization and does not account for unexpected anatomical variations. This may lead to treatment failure. ECP performed in glaucomatous eyes previously treated with TCP provides additional IOP control since it allows direct treatment of previously skipped ciliary processes and in between the processes.

Keywords: Glaucoma, cyclophotocoagulation, intraocular pressure, endoscopic cyclophotocoagulation

ABSTRACT #8

THE ZINC CHELATOR TPEN IS A SELECTIVE INDUCER OF AXIDATIVE STRESS AND APOPTOSIS IS HUMAN COLON CANCER CELLS

Maamoun Fatfat[1], Angela Zaki[1], Hazar Haidar[1], Ghizlaine Bendriss[2], Khaled Machaca[2], Hala Gali-Muhtasib[1*]

Department of Biology[1], American University of Beirut, Lebanon, Department of Physiology and Biophysics[2], Weill Cornell Medical College, Qatar
*Corresponding Author: amro@aub.edu.lb
Maamoun Fatfat is a Postdoc at the Biology Department.

Background and Aims
The cell permeable and high affinity zinc chelator N, N, N', N' tetrakis 9-(2-pyridylmethyl) ethylenediamine (TPEN) has been shown to induce apoptosis in many cancer cell lines; however, its mechanism of cell death is not defined yet. The aim of the paper is to show that TPEN selectively induces apoptosis in cancer but not in normal cells due to its ability to generate higher ROS levels in the former cells based on their higher intrinsic metal content.

Methods
HCT116 $p53^{+/+}$ human colon cancer cells were cultured and were treated with TPEN at 50% confluency. The cell viability assay used was an MTT –based method. Cell cycle analysis was performed using flow cytometry. Other assays used include: TUNEL Assay, Annexin V Assay, Zinquin Assay, and DCFH Assay, evaluation of mitochondrial transmembrane potential, caspase activity assays, and western blotting. Mouse xenografts were also performed to check the drug's efficacy.

Results
Treatment of HCT116 colon cancer cell lines with as low as 1µM TPEN reduced cell viability and induced apoptosis, while normal human colon NCM 460 and human intestinal FHS74Int were not affected by 5-fold higher concentrations. In HCT116 cells, TPEN caused the loss of mitochondrial membrane permeability, release of cytochrome c, activation of caspases-3 and -9 and cleavage of poly ADP ribose polymerase (PARP). Apoptosis by TPEN correlated with the degradation of the X-linked inhibitor of apoptosis (XIAP), which occurred by proteosomal-dependent mechanisms. Reactive oxygen species (ROS) generation was found to play the most important role in TPEN's apoptotic mechanisms, and to explain the differential sensitivity of cancer vs. normal cell lines. In comparison to NCM460 cells, HCT116 cells were found to contain 5-10 fold higher intrinsic levels of zinc and copper, two metals responsible for the production of ROS.

Conclusion
The selectivity of TPEN to colon cancer cells suggests its potential therapeutic role against colon cancer.

Keywords: Zinc chelator, TPEN, colon cancer, apoptosis, ROS

ABSTRACT #9

BONE IMAGE SEGMENTATION USING NEURAL NETWORKS

I.Hage [1], R.Hamade [2]

1American University of Beirut (AUB), Department of Mechanical Engineering, PhD candidate, ish07@aub.edu.lb.
2: American University of Beirut (AUB), Department of Mechanical Engineering, Professor, rh13@aub.edu.lb.

Introduction
Background: In medicine, image processing by electronic means has been a very active field for decades, due to its positive contribution in accurate medical analysis. Segmentation of medical images is an important link in image processing and pattern recognition. The goal has been, and still is, to have a machine perform the same image functions which humans do quite easily. Pulse coupled neural networks (PCNN) is a pre-processing algorithm recently developed for image segmentation and it shows tremendous promise to achieve this goal.

Aims
The PCNN is a novel neural network algorithm that produces a series of binary pulse images when stimulated with a grey scale or color image. This algorithm was used in this project to segment bovine (cow) femur bone images at the micro scale, with target to identifying micro-features of small spatial extent at low signal to noise ratio.
At the micro scale a cortical bone is comprised of haversian canals (where blood runs), lamellae surrounding haversian canal, both concentric lamellae and the canal form the osteon which are separated from each other by cement lines, and all these features are distributed in an interstitial matrix.

Methods
When PCNN is used, its parameters aren't self-adapting according to different image, this project propose a new method based on particle swarm optimization (PSO) and adaptive threshold, to determine automatically the parameters of PCNN.

Results
The image entropy (H) was extracted using training data for each micro constituent and the segmentation/classification of the bone features was based on this vector/feature.

Conclusion
Results demonstrate that the proposed method is accurate and robust for bone image segmentation.

Keywords: Bone image, segmentation, neural networks, optimization.

ABSTRACT #10

MECHANISM OF ACTION OF THE SYNTHETIC RETINOID ST1926 IN CHRONIC MYELOID LEUKEMIA

R. Hmadi,[1] *A. Iskandarani,[2] R. Nasr,[2] and N. Darwiche [1]*

[1]Departments of Biochemistry and Molecular Genetics, [2]Anatomy, Cell Biology, and Physiology, American University of Beirut, Beirut, Lebanon
*Emails: - Corresponding Author: Dr. Nadine Darwiche (nd03@aub.edu.lb)
- Research Assistant: Raed Hmadi (rh112@aub.edu.lb)

Background and Aims
Chronic Myeloid Leukemia (CML) is a clonal hematopoietic stem cell myeloproliferative disorder caused by the formation of the Philadelphia chromosome, due to a balanced translocation between chromosomes 9 and 22 and the formation of the *bcr-abl* fusion gene that encodes the BCR-ABL oncoprotein with constitutive tyrosine kinase activity. Imatinib, a tyrosine kinase inhibitor, is the first line of treatment for CML patients worldwide. Unfortunately, several patients develop resistance to imatinib which necessitates the search for novel treatments. Retinoids regulate crucial biological processes such as cellular proliferation, apoptosis, and differentiation, in particular of hematopoietic progenitor cells. All-*trans* retinoic acid (ATRA) is a naturally occurring retinoid used as an anticancer agent. Unfortunately, the clinical usage of natural retinoids is hindered by undesirable side effects and acquired resistance. Therefore, synthetic retinoids, such as ST1926, which couple increased specificity and reduced toxicity were developed.
We investigated the effects of ST1926 on i) the proliferation of human CML cell lines (AR230, K562, and LAMA), ii) cell-cycle progression and apoptosis induction, iii) the activity of constitutively active oncoprotein BCR-ABL and downstream targets, and iv) whether ST1926 synergizes with imatinib to inhibit the growth of human CML cell lines.

Methodology and Results
Using the MTT cell proliferation and trypan blue exclusion assays, we have shown that all tested CML cell lines are resistant to ATRA. Interestingly, these CML cell lines were sensitive to physiologically achievable micromolar concentrations of ST1926, while normal lymphocytes were not affected up to ten-fold higher supraphysiological concentrations. ST1926 induced apoptosis, as shown by the accumulation of cells in the pre G_1 region of the cell cycle and TUNEL positivity. Most importantly, ST1926 reduced BCR-ABL protein levels and activity, along with the downstream mediator STAT5. Interestingly, combined ST1926 treatment with imatinib resulted in additive growth inhibition of all tested CML cell lines.

Conclusion
These results highlight the anti-tumor properties and potential therapeutic activity of ST1926 in CML cells. We are currently investigating the anti-tumor activities of ST1926 in a well-established CML mouse model.

Keywords: chronic myeloid leukemia, retinoids, ST1926, apoptosis, BCR-ABL.

ABSTRACT #11

CONNEXIN43 OVER-EXPRESSION INDUCES PARTIAL MESENCHYMAL-EPITHELIAL TRANSITION IN MDA-MB-231 BREAST CANCER CELLS

Elia T. El Habre[a], Marwan E. El-Sabban[b] and Rabih S. Talhouk[a]

Departments of [a]Biology, Faculty of Arts and Sciences and [b]Anatomy, Cell Biology and Physiology*, Faculty of Medicine, American University of Beirut. Beirut, Lebanon.*
*Email: ete00@aub.edu.lb
Position: Masters Student

Introduction
Many studies have classified cancer in the category of connexin-related pathologies. Addressing the role of connexins in breast cancer progression has yielded contradictory results. While connexins have long been considered as tumor suppressor genes, other data indicate that invasive breast carcinomas express high levels of connexins.

Methods
In order to investigate the role of Cx43 in highly invasive breast cancer cells, we over-expressed Cx43 in MDA-MB-231 cells, a human breast cancer cell line that expresses low endogenous Cx43 levels.

Results
Our data shows a decrease in invasion, motility and cell proliferation and cell-cycle modulation in Cx-43 overexpressing cells grown in 3D cultures on Matrigel, a milieu that more faithfully mimics the *in vivo* context. The decrease in cell proliferation and cell-cycle modulation were not noted in cells grown on 2D. This effect is mediated, in part, by exogenously expressed Cx43 interacting with its associated proteins and assembling at the membrane in 3D, but not in 2D, cultures. As opposed to sham and untransfected cells, qRTPCR of Cx43 overexpressing cells showed a 2-fold decrease in Zeb1/GAPDH mRNA levels in 3D but not in 2D cultures. A similar trend was also noted in vimentin/GAPDH levels, but in both 2D in 3D cultures. Interestingly both protein and mRNA levels of Twist1 were unaltered across conditions. The data suggests that Cx43 mediates a selective transcriptional downregulation of certain Epithelial-Mesenchymal Transition markers, and this correlates with a less invasive phenotype of MDA-MB-231 cells.

Conclusion
In conclusion, Cx43 alters tumor phenotype of highly metastatic breast cancer cells in a context dependent manner and partially induces Mesenchymal-Epithelial Transition.

Keywords: Epithelial-Mesenchymal Transition (EMT), Breast Cancer, Connexin43

ABSTRACT #12

EFFECT OF CONNEXIN 43 LOSS ON POLARITY AND INITIATION OF TUMORIGENIC PATHWAYS IN THE PHENOTYPICALLY NORMAL MAMMARY EPITHELIUM

Bazzoun, D.B.[1,2], Adissu, H.A. [2], Labban, D.L.[1], Nahas, R.N.[1], Sabban, M.S.[1], Lelièvre, S.A.[2], and Talhouk, R.S.[1]

1- Department of Biology, Faculty of Arts and Sciences, American University of Beirut (AUB), Beirut, Lebanon; 2- Department of Basic Medical Sciences and Center for Cancer Research, Purdue University, West Lafayette, IN, U.S.A.
*Email: dab05@aub.edu.lb, dbazzoun@purdue.edu
Ph.D. Student-Cell and Molecular Biology Program at the Biology Department-AUB

Introduction
Recent evidence suggest a regulatory role for Connexin (Cx) 43, a gap junction (GJ) protein, in apical polarity establishment, Apical polarity is established via the localization of tight junction complexes at the apex of epithelial cell-cell contacts, against the lumen in which milk is secreted. It is a key property of epithelial tissues that is disrupted early on during tumorigenesis and is necessary for cells to enter the cell cycle, a prerequisite of cell proliferation. However, the mechanism that governs Cx43 role in regulating apical polarity of the mammary epithelium is yet to be revealed. We have demonstrated a Cx43 context dependent tumor-suppressive role mediated partially by a GJ complex assembly that sequesters β-, α-catenin and ZO-1 proteins at the cell membrane of breast epithelial tumor cell lines. We hypothesize that the absence of Cx43 in the non-neoplastic epithelium affects pathways involved in tumor initiation.

Methods
For this purpose, we stably silenced Cx43 using a Cx43 specific shRNA and a non-specific (NSS) shRNA in HMT-3522 S1 non-neoplastic breast epithelial cells.

Results
Cx43-shRNA cells showed a 35% increase in their proliferation rate on day 8 in flat monolayer (2D) culture in comparison to the NSS-shRNA cells. Following the use of 3D cell culture that permits the formation of physiologically relevant epithelial glandular structures (acini), we measured a 38% increase in the size of S1 acini with silenced Cx43 compared to control NSS-shRNA acini. Moreover, silencing Cx43 disrupted epithelial polarity by mislocalizing both ZO-1, a binding partner of Cx43 and a tight junction associated protein, and β-catenin, a binding partner of Cx43 and a key protein in proliferation pathways, as revealed by immunostaining. β-catenin total protein levels were also up-regulated in Cx43-shRNA S1 cells as revealed by immunoblotting and a misdistribution of this protein was observed. Furthermore, Cx43 silencing induced a down-regulation and a mislocalization in protein expression of *SCRIB*, a key regulator of apical polarity and a tumor suppressor. Similar preliminary results were obtained in silenced Cx43 MCF-10A cells, except for MCF-10A cells lumen formation was disrupted in contrast to HMT-3522 S1 cells.

Conclusion
These results reveal an intimate link in the breast epithelium between Cx43 and several components of the apical pole of epithelial cells that have been involved in tumor onset.

Keywords: Connexin 43, Gap Junction, Apical Polarity, Mammary Epithelial Cell

ABSTRACT #13

DCQ IS A HYPOXIA-ACTIVATED DRUG THAT INDUCES ROS-MEDIATED APOPTOSIS AND REDUCES BREAST CANCER METASTASIS IN VITRO AND IN VIVO

Khaled Ghattass[1], Sally El-Sitt[1], Kazem Zibara[2], Saide Rayes[3], Makhlouf M.J. Haddadin[3], Marwan El-Sabban[Ψ4] and Hala Gali-Muhtasib*[1]

1) Department of Biology, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon.
2) Department of Biology, Doctoral School of Science and Technology (DSST), Lebanese University, Beirut, Lebanon.
3) Department of Chemistry, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon.
4) Department of Anatomy, Cell Biology and Physiological Sciences, Faculty of Medicine, American University of Beirut, Beirut, Lebanon.
*Advisor: Dr. Hala Gali-Muhtasib
Ψ Co-advisor: Dr. Marwan El-Sabban
*Email: Khaled Ghattass: kig01@aub.edu.lb

Background and Aims
Although tumor hypoxia poses significant challenges against conventional cancer treatments, it provides a target for therapy by hypoxia-activated drugs. Here, we study the effect of the hypoxia-activated synthetic quinoxaline di-N-oxide (DCQ) on breast cancer progression and identify molecular mechanisms involved in DCQ activity.

Methods
To address our aims we have used human breast cancer cell lines (MDA-MB-231, and MCF-7, DCQ, Hypoxia chamber, MTT proliferation assay, colony formation assay, flow cytometry of PI stained DNA and DCFH assay, ELISA of secreted VEGF, Western blot analysis, and xenograft model of subdermally injected MDA-MB-231 cells in immune-compromised mice.

Results
DCQ induces reactive oxygen species (ROS)-dependent p53-independent apoptosis in MDA-MB-231 and MCF-7, preferentially under hypoxia, an effect that was inhibited by antioxidants. DCQ-induced ROS was associated with DNA damage and inhibition of hypoxia inducible factor (HIF-1α) accumulation. The inhibition of HIF-1α in MCF-7 was in part *via* the activation of p53 and was accompanied by a decrease in the levels of phosphorylated mTOR, indicating that DCQ is acting at the translational level of the protein. In MDA-MB-231 cells, DCQ reduced HIF-1α through proteasomal degradation. Interestingly, HIF-1α reduction led to reversal of the hypoxia-induced VEGF secretion and invasion in MCF-7 and to the reduction of TWIST in MDA-MB-231. Moreover, DCQ significantly increased the survival of immune-compromised mice subdermally injected with MDA-MB-231 cells and reduced metastatic dissemination of breast cancer cells into their lungs and liver.

Conclusion
The ability of DCQ to induce ROS-dependent apoptosis, inhibit HIF-1α in two distinct mechanistic routes in MCF-7 and MDA-MB-231 cells, and to reduce metastatic dissemination suggests that this molecule has promising anticancer effects and should be moved further to clinical investigation.

ABSTRACT #14

ETIOLOGY AND CLINICAL FEATURES OF A COHORT OF PATIENTS WITH OCULAR INFLAMMATION IN LEBANON

Mahmoud O Jaroudi, M.D. (chief resident), Marwan Abdel Aal, M.D., and Rola Hamam, M.D.
Department of Ophthalmology, American University of Beirut; Beirut, Lebanon
Reprint request: Rola Hamam, M.D., Department of Ophthalmology, American University of Beirut, Beirut, Lebanon,
*Email: rh46@aub.edu.lb

The authors declare no conflict of interest.

Purpose
Study the etiology and clinical features of ocular inflammatory diseases in patients seen in the uveitis clinic at the American University of Beirut Medical Center.

Methods
The charts of 244 patients seen between January 2009 and September 2011 were reviewed. Among 244 patients, 216 patients had uveitis and 28 patients have other ocular inflammation. Data pertaining to patients' age, gender, location of the inflammation (anterior, posterior, intermediate & panuveitis), pathogenesis, and presence of systemic disease were collected.

Results
The mean age at presentation was 36.9 years (2-85). Male to female ratio was 1:1.2. 151 patients (71.2%) had noninfectious uveitis. The most common presentation was panuveitis (43%), followed by anterior uveitis (26%), posterior uveitis (21.2%), then intermediate uveitis (9.2%).
28.8% patients had infectious etiology, 32% had idiopathic, 30,6% were associated with systemic etiologies, and 5.5% had specific ocular conditions. 3 patients (1.5%) had masquerading malignancies.
The most common infectious entities were herpetic anterior uveitis, toxoplasmosis, and CMV followed by tuberculosis. The most common identifiable non-infectious entity was Behçet's disease. HLA-B27 acute anterior uveitis, ocular sarcoidosis, juvenile idiopathic arthritis associated uveitis were common. The mean duration of the disease before presenting to the tertiary center was 34 months. 27% of the patients had worse than 20/200 vision in their involved eye upon presentation. 35% of the patients had cataract and 11% were pseudo-phakic upon presentation.

Conclusion
Panuveitis was the most common presentation. Idiopathic and autoimmune inflammation were the most common etiologies. Definitive diagnosis could be established for 71 % of patients. Analysis of 30% of the data revealed that patients tend to be referred to tertiary center after long time of the beginning of their disease (34 months).

Key words: Idiopathic uveitis; infectious uveitis, ocular inflammation

ABSTRACT #15

SEA CUCUMBER EXTRACTS SELECTIVELY INHIBIT THE PROLIFERATION OF NORMAL RODENT MAMMARY CELLS AND PARTIALLY REVERT THE TUMOR PHENOTYPE OF BREAST CANCER CELLS

Kareh Mike[1*], Al-Ghadban Sarah[2*], Nahas Rana[*], Al-Araj Lamis[3*], Saliba Najat[3*], El-Sabban Marwan[2*], Talhouk Rabih[1*]

*Department of [1]Biology, [3]Chemistry, Faculty of Arts and Sciences, [2]Anatomy, Cell Biology and Physiology, Faculty of Medicine, and *IBSAR-Nature Conservation Center (NCC) for Sustainable Futures, American University of Beirut, Lebanon.*
*Email: Mike Kareh; mik16@aub.edu.lb (Student) and Rabih Talhouk; rtalhouk@aub.edu.lb (professor)

Introduction
The unexploited and rich biodiversity of the Mediterranean Sea marine life has recently attracted the interest of researchers seeking natural bio-active compounds. Currently, we are investigating the presence of anti-inflammatory and anti-cancer bioactivities in *Holothuria polii*, the most abundant species of sea cucumber inhabiting the Mediterranean.

Methods
In order to test for bioactivity, first we extracted the whole sea cucumber in ethanol and the extract was tested on human rodent mammary SCp2 cells and on human breast cancer MDA-MB-231 cells to check for anti-inflammatory and anti-cancer activity respectively.

Results
Ethanol extract of whole sea cucumber inhibited the growth of rodent mammary SCp2 cells without affecting other cellular functions such as the production of basal levels of inflammatory markers, namely interleukin 6 (IL-6), Nitric Oxide (NO) and Matrix Metalloproteinase 9 (MMP9). Furthermore, no change in endotoxin (ET) induced levels of IL-6, NO and MMP9 have been detected after treatment of SCp2 cells with cucumber extract. Treatment of breast cancer MDA-MB-231 cells with cucumber extracts inhibited the growth of these cells in both, 2D cultures on plastic and 3D cultures on matrigel, by 60% on day 5 of culture. MDA-MB-231 cells, when cultured on matrigel cluster into spherical aggregates with stellate outgrowths and only 10% to 20% form spherical aggregates lacking stellate outgrowth. Treatment with cucumber extracts shifted the percentage of stellate clusters from 80% to 40% and favored spherical aggregate morphology which more closely resembles the growth of normal breast epithelial cells on matrigel. Moreover, RT-qPCR showed a decrease in the mRNA levels of the mesenchymal markers ZEB1 and TWIST by 30% and 80% respectively. Preliminary data from a bio-guided fractionation study showed that the aqueous but not the butanol fraction retained the anti-proliferative activity detected in the whole extract. The partially purified aqueous fraction inhibited the growth of SCp2 cells by 50% on day 5 of culture.

Conclusion
We speculate that this bioactivity selectively affects cellular processes related to proliferation and mesenchymal epithelial transition. Further studies will be conducted to determine the characteristics of this selective activity.

Keywords: Sea Cucumber, anti-inflammatory, anti-proliferative.

ABSTRACT #16

INNOVATIVE CHARGE DELIVERY TECHNIQUES IN ELECTROCONVULSIVE THERAPY

Mohamad Awada, Karim Zahed, Firas Mourad, Dr. Fadi Karameh

Associate Professor of ECE
Undergraduate CCE students
*Email: mohammed.awada@gmail.com

Introduction

The project aims at creating a simulator for the electroconvulsive therapy (ECT) procedure. ECT is a treatment for severe depression and the procedure works by inducing seizures in the focal brain areas (cortical region especially). The seizures are induced by applying currents through electrodes placed on the head.

Methods

The simulator will combine the boundary element modeling of OpenMEEG with the computational power of MATLAB. OpenMEEG only models passive elements, so our simulator will create active elements by using a bidirectional current. If successful, the simulator may be used by the Department of Psychiatry at AUBMC so that psychiatrists will be able to know the effect of using a specific electrode configuration or current strength or pulse shape on different parts of the brain. We also hope to create an interactive visual representation of the result. Once the simulator is finished, we will be able to test some statistical properties of neuronal models in the spring semester.

In order to do this, the group had to understand some underlying mechanisms in the brain, especially those involved with seizures. Until now, we have modified the Wendling neuronal model and implemented it on MATLAB. The purpose of the modification was to allow a seizing network to drive a non-seizing network into seizure.

The approach taken in modifying the model was first theoretically adding links to make multiple circuits respond realistically to external stimulation. Then we introduced the changes into the differential equations involved. The links introduced were from the output of the slow inhibiting dendrites to the input of the fast-inhibiting soma, from the output of one network to the input of the other and from the output of one network to the input of the slow-inhibiting dendrites in the other. After tweaking the differential equations, we ran simulations to sweep across the multiplicative factors involved with the links to understand the power of the links introduced and find the proper range of multiplicative factors to bring the networks to seizure. The next step is to integrate the modified Wilson model with the OpenMEEG boundary element model to get a functional simulator.

Results and conclusion

This project is still in progress, and the simulator should be finished by mid-April.

Keywords: ECT, seizure, neuronal populations

ABSTRACT #17

CARCINOGENS IN WATERPINE (NARGHILE) SMOKE

Elizabeth Sepetdjian[a], Rola Salman[b], Alan Shihadeh[b], Najat Saliba[a]

a Atmospheric and Analytical Laboratory, Chemistry Department, AUB, Beirut, Lebanon
b Aerosol Research Laboratory, Mechanical Engineering Department, AUB, Beirut, Lebanon
*Emails: Corresponding Authors: *Najat A. Saliba, e-mail: ns30@aub.edu.lb*
Alan Shihadeh, e-mail: as20@aub.edu.lb
Elizabeth Sepetdjian: Research Associate

Introduction
The recent rise in the popularity of the narghile waterpipe in regions where it is culturally rooted, as well as in Europe and North America has been attributed to the marketing and availability of ma'assel, a heavily sweetened and flavored tobacco mixture. Selected Toxins analyzed in waterpipe smoke and compared to mainstream cigarette smoke are: nicotine, NO, CO, polycyclic aromatic hydrocarbons (PAHs), aldehydes and phenols. Some of these toxins are also quantified in the sidestream smoke and are considered major contributors to the environmental tobacco smoke.

Methods
Depending on the chemical properties of each family of compounds, different analytical techniques were developed to quantify the different toxins in the waterpipe standard and human-mimic smoking sessions.

Results
Studies show that serious health implications are evident for waterpipe smokers. Compared to a single cigarette tested, waterpipe smoking session delivers about 20 times the total PAH yields, 50 times the heavy (4- and 5-ring) PAHs listed as class 1 carcinogens by the International Agency for Research on Cancer (IARC). Also one waterpipe session equals to 17 cigarettes in formaldehyde (class 1 carcinogen), five cigarettes in acetaldehyde, three cigarettes in seven standard phenol compounds and 1000 cigarettes in phenols' derivatives and flavorings such as methylated dihydroxybenzenes, vanillin, ethyl vanillin, and benzyl alcohol. This is due to many factors affecting the formation of these toxins in the waterpipe smoke such as; amount and type of tobacco, presence of charcoal as a heating source, the low temperature at which the tobacco is heated, the amount of total particulate matter delivered.

Conclusion
Our results prove the misperception among smokers that the water through which the smoke bubbles filters the toxic components rendering the practice considerably less harmful than cigarette smoking.

Keywords: Waterpipe smoke; Aldehydes; PAH; Ma'assel tobacco; Carcinogens

ABSTRACT #18

DEVELOPMENT AND CHARACTERIZATION OF THYMOQUINONE NANOPARTICLES FOR CANCER THERAPY

Isabelle Fakhoury[1], Kamal Bou Hadir[2], Walid Saad[3*], Hala Muhtasib[1, 4*]

Departments of Biology[1], Chemistry[2], Chemical Engineering[3] and AUB Nature Conservation Center[4], American University of Beirut, Lebanon
*Emails: amro@aub.edu.lb, ws20@aub.edu.lb
Isabelle Fakhoury, PhD student in Cell and Molecular Biology

Background and Aims
Thymoquinone (TQ) is a hydrophobic natural product that has shown promising anticancer properties in pre-clinical studies. The poor water solubility of anticancer drugs however, has been known to affect their bioavailability *in vivo* and thus their activity. Polymeric nanoparticle (NP) delivery system has emerged as a solution for such problem as it can stabilize, protect, and allow controlled release of the drugs as well as increase their oral bioavailability and overall efficacy. To this aim, we sought to design TQ-NPs, characterize them as well as assess their antitumor potential *in vitro* and *in vivo*.

Methods
TQ nanoparticles (TQ-NPs) were prepared by flash nanoprecipitation using a confined volume impinging jet and controlled flow rates as described by Johnson and Prud'homme in 2003. The formulation was composed of TQ, the amphiphilic diblock polymer poly(styrene-b-ethylene oxide) (PS(1500)-PEO(2400)) (PSEO) in a weight ratio of 1:1 at a concentration of 50 mg per ml of tetrahydrofuran (THF) solvent. The size of the TQ-NPs was measured by dynamic light scattering (DLS) and TQ amounts were quantified by High Performance Liquid Chromatography (HPLC). The TQ-NPs stability was monitored over 1 month by both DLS and HPLC analysis. The NPs were further characterized by measuring their entrapment efficieny as well as by following their controlled release behavior *in vitro*. Finally, Trypan blue assay was used to assess cell viability of Jurkat and HuT-102 leukemia cells *in vitro* after treatment with free TQ or TQ-NPs.

Results
We have succeeded in formulating TQ-NPs with effective diameter size ranging between 40-50 nm. The obtained NPs therefore conform to the National Nanotechnology Initiation recommendations for particle size. Stored at room temperature, the TQ-NPs were stable for 1 month. *In vitro*, both Jurkat as well as HuT-102 cells lines were more sensitive to TQ-NPs as compared to free TQ while no significant effect was noted in response to treatment with blank NPs containing the PSEO polymer alone.

Conclusion
Our data highlight a promising antitumor potential for TQ-NPs.

Keywords: Thymoquinone, nanoparticles, cancer therapy

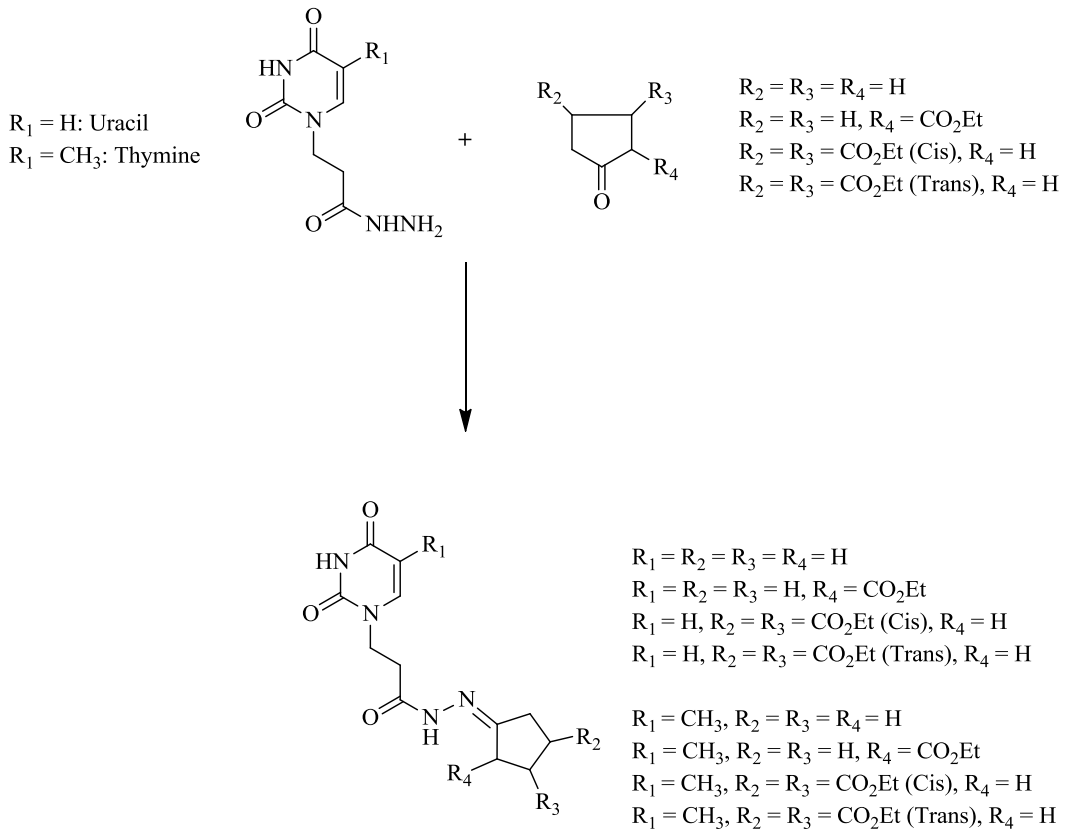
ABSTRACT #19

SYNTHETIC PYRIMIDINE-BASED NUCLEOSIDES

Nourhan Chaaban, Rima Hajjar, Ali Koubeissi, Assaad Eid and Kamal Bouhadir

Department of Chemistry, Faculty of Arts and Sciences, AUB
Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine, AUB
Undergraduate students at the Biology department at AUB.

Carbocyclic nucleosides (carbanucleosides) displayed a wide range of biological activities up to date including antitumor, antibiotic, antimicrobial, antiviral, antimetabolite and herbicidal activities as well as inhibition of S-adenosyl-L-homocysteine hydrolase and picornavirus. One attractive feature in carbocyclic nucleosides is the replacement of the furanose ring with a cycloalkane ring that is resistant against phosphorylases that cleave the *N*-glycosidic bond in neutral nucleosides. In this study, we report the synthesis of a new series of carbocyclic uridine and thymidine hydrazones to evaluate their effect on high-glucose (HG)-induced mesangial cells proliferation and HG-induced fibronectin expression. The synthesis of these hydrazones involves a coupling reaction between cyclopentanone derivatives and 1-(2-hydrazidoethyl)uracil or 1-(2-hydrazidoethyl)thymine.



Key Words: Modified nucleosides, Carbanucleosides, Uracil, Thymine, Hydrazones.

ABSTRACT #20

THE EFFECT OF HIGH FAT MEAL CONTAINING PHOSPHORUS ON POSTPRANDIAL LIPID STATUS OF HEALTHY SUBJECTS

J. Hazim (MSc student), D. Shatila, H. Ghattas, S. Hlais, O. Obeid

M. Khattab, D. dit El-Khoury, S. Azar, M. Mattar and O. Obeid. Effect of phosphorus on the oral glucose tolerance test. Proceedings of the Nutrition Society (2011), 70 : E60
Department of Nutrition and Food Sciences, American University of Beirut, Lebanon
Conflict of interest: None
Funding: University Research Board, American University of Beirut
*Email: Corresponding author: omar.obeid @aub.edu.lb

Introduction

Phosphorus ingestion is known to affect insulin sensitivity (Khattab *et al* 2011) and it is not clear whether such effect can impact postprandial lipid status.

Methods

A randomized cross over study was conducted, in which overnight fasted healthy subjects (8 males) received high fat meal [330kcal; Energy (%): 69 fat, 28 CHO, 3 protein] with or without phosphorus (500mg). Blood samples (Baseline, 1, 2, 3, 4, 5 and 6 hr after meal ingestion) were collected and plasma concentration of total Phosphorus, Insulin, Triglyceride (TG), ApoB48 and non-esterified free fatty acids (NEFFA) were determined. Changes in metabolite's concentration from baseline were analysed using two-way analysis of variance.

Results

Ingestion of high fat meal containing phosphorus was associated with a statistically significant change in total phosphorus. The changes in plasma insulin and TG concentration were similar between the two meals, while that of NEFFA was significantly different according to time only. However, the changes in plasma ApoB48 was significantly different according to phosphorus content of the meal, in which that of phosphorus containing meal was higher and sustained for longer duration.

Conclusion

The level and duration of postprandial plasma ApoB48 was found to be increased in the phosphorus containing high fat meal.

Keywords: Phosphorus, lipid profile, human

ABSTRACT #21

PRO-ANGIOGENIC FACTORS AND HETERO-CELLULAR INTERACTION IN CANCER CELLS METASTASIS

Kazem Zibara[1], Zahraa Awada[1], Marwan El-Sabban[2]

[1] Department of Biology, Faculty of Sciences and EDST, Lebanese University
[2] Department of Anatomy, Cell Biology, and Physiology, Faculty of Medicine, American University of Beirut
Participant: Kazem Zibara

Introduction
Metastatic spread of cancer cells is the most devastating aspect of malignancy. Cancer cells secrete VEGF, which plays a key role in the growth, neovascularization, invasion, extravasation and metastasis of cancer cells. Equally, of critical importance in the process of extravasation, is the direct cancer cell-endothelial cell interaction mediated by gap junctions. In this study, we evaluate the anti-angiogenic, anti-tumor and the anti-metastatic activities of Avastin (Av), an anti-VEGF antibody; and Oleamide (OL), a gap junction inhibitor, using MDA-MB-231 human breast cancer cells *in vitro* and in an *in vivo* xeno-graft murine model.

Methods
The effect of Av, OL or their combination on proliferation, cell cycle, migration and invasion, and intercellular communication was studied by western blots, gelatin zymography, RT-PCR, flow cytometry and ELISA. Several metastatic markers including Cx43, Cx26, VEGF, MMPs, HIF1α and CXCR4 were evaluated. The *in vivo* anti-tumor effect and survival studies of Av/OL were determined by subcutaneous and intravenous injections of MDA-MB-231 cells in NSG mice. The effect of Av/OL on pulmonary and hepatic metastases was assessed by H and E staining, western-blots and RT-PCR.

Results
Av/OL significantly decreased proliferation, induced cell cycle arrest and attenuated invasion and migration of MDA-MB-231 cells *in vitro*. In addition, the expression levels of key mediators of metastasis such as Cx43, MMP-2, MMP-9, VEGF, HIF1α and CXCR4 decreased upon Av/OL treatment *in vitro*. Moreover, avastin, but not oleamide, reduced tumor size of MDA-MB-231 cells injected in NSG mice, which was also associated with increased mice survival. Finally, both drugs reduced pulmonary and hepatic metastatic foci *in vivo*.

Conclusions
While Avastin has anti-angiogenic, anti-tumor and anti-metastatic activities, Oleamide has anti-metastatic activity, presumably at the extravasation level, providing further evidence for the role of GJIC in the accomplishment of cancer cell extravasation.

Keywords: Extravasation, VEGF, Gap Junction, Connexins, GJIC

ABSTRACT #22

CONTROL OF ADULT NEURAL CELLS (NSCs) BY THE RETINOBLASTOMA PROTEIN, pRb IN VITRO

HAYEK Dayana, SAAB Raya and GHANEM Noël*

[1]Department of Biology, AUB
[2]Department of Pediatric and Adolescent Medicine, AUBMC
*Corresponding author: ng13@aub.edu.lb

Introduction
During development, the tumor suppressor gene, pRb, regulates distinct aspects of neurogenesis including neuronal proliferation, differentiation and migration. We recently investigated the role of Rb in adult neurogenesis and showed that it specifically controls proliferation of neural stem cells (NSC) and progenitors in the adult subventricular zone (aSVZ) without affecting their differentiation program. Here, we examined the properties of Rb-null NSCs *in vitro* including their self-renewal capacity and differentiation potential and compared them with Rb-wt NSCs.

Methods
We induced a temporal deletion of Rb in 8 week-old mice using a Nestin-CreERT2-YFP tamoxifen-inducible system and $Rb^{floxed/floxed}$ mice. 10 days following treatment, we dissected and dissociated the adult SVZ tissue, then performed neurosphere assay. Cells were plated at low and high densities in media supplemented with FGF2 and EGF, then cultured for 7 days.

Results
We assessed the number and the size of primary neurospheres derived from Rb-null versus Rb-wt NSCs/progenitors and found a ~3 fold increase in the total number of spheres generated in the absence of Rb compared with controls. These neurospheres had also a larger size compared with controls on average. Next, we examined the self-renewal capacity of Rb-null NSCs and found that they exhibited a higher self-renewal potential after 2 passages (secondary neurospheres) compared with wt-NSCs. We are presently testing the multipotency of Rb-null NSCs/progenitors by conducting differentiation assays.

Conclusion
Consistent with our *in vivo* results, our findings demonstrate that Rb controls the rate of proliferation and self-renewal capacity of adult NSCs *in vitro* which may have direct implications for the use of these cells in regenerative medicine. *Supported by grants from URB and LNCSR*.

Keywords: Rb, adult neurogenesis, neural stem cells, neurosphere assay

ABSTRACT #23

ROLE OF THE RETINOBLASTOMA PROTEIN, pRb, IN THE DEVELOPMENT OF THE OLFACTORY SYSTEM

JAAFAR Carine, AL LAFI Sawsan and GHANEM Noël*

Department of Biology, AUB
*Email: ng13@aub.edu.lb

Introduction

The retinoblastoma, pRb, is a tumor suppressor gene that plays important roles in brain development primarily by controlling cell division at the G1-S phase checkpoint. In addition, loss of Rb causes neuronal differentiation and migration defects in the developing brain. We investigated here the role of Rb in the development of the olfactory system (OS) which is comprised of the olfactory epithelium (OE) and the olfactory bulb (OB). We analyzed, in the absence of Rb, the morphogenesis of the OS and studied the development of olfactory sensory neurons (OSN) and the olfactory nerve layer (ONL).

Methods

We performed a conditional Rb deletion in the telencephalon and OS using Foxg1-Cre mice and $Rb^{floxed/floxed}$ mice, and, used cresyl-eosin staining and immunohistochemistry to examine and compare the OS phenotype in Rb-null mice versus controls between E15.5 and birth.

Results

Neurogenesis and synaptogenesis in the OS are regulated by reciprocal interactions between the OE and the OB during development. We assessed both of these processes and found that loss of Rb leads to: 1) layer disorganization and hypoplasia in the OB, 2) ONL defects manifested by abnormal projections of OSN axons toward the bulb, 3) aberrant maturation of OSN associated with ectopic localization in the basal side of the OE, and, 4) increased cell death in both the OB and OE with gradual degeneration of the latter around birth.

Conclusion

Our data shows clearly that Rb is required for normal development of the olfactory system and emphasizes a novel role for this cell cycle protein in morphogenesis and the establishment of appropriate neuronal connections between different brain regions. *Supported by grants from URB and LNCSR*.

Keywords: Rb, olfactory epithelium, olfactory bulb, neurogenesis, synaptogenesis

ABSTRACT #24

CLN3, A NOVEL MOLECULAR TARGET FOR BREAST CANCER

Joelle Makoukji[1], Mohamad Raad[1], Katia Genadry[1], Arafat Tfaily[3], Fouad Boulos[4], Rose-Mary Boustany[1,2]

[1]Department of Biochemistry and Molecular Genetics, [2]Neurogenetics Program and Division of Pediatric Neurology, Department of Pediatrics and Adolescent Medicine, [3]Department of Internal Medicine, [4]Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Corresponding author: Joelle Makoukji - postdoctoral fellow *E-mail: jm53@aub.edu.lb

Introduction

Breast cancer (BC) is the most common cancer in women. Elucidation of underlying molecular pathways is necessary to personalize therapy and improve outcomes. CLN3p is anti-apoptotic, and defects in *CLN3* cause accelerated apoptotic death of neurons/other cells in juvenile Batten disease. CLN3p is upregulated in many cancer cell lines, including human/murine breast cancer cell lines. Dysregulated apoptotic pathways are implicated in development of the oncogenic phenotype. One **aim** is establishing *CLN3* expression in the breast cancer cell line, MCF7, and determining effect of *CLN3* levels on cell growth/apoptosis. A second **aim** is determining expression of *CLN3* in normal vs. tumor samples from fresh and formalin-fixed/paraffin-embedded (FFPE) breast tissue, and documenting subcellular localization of CLN3p in *CLN3*-overexpressing cancerous vs. normal breast tissue.

Methods

CLN3 expression was determined by RT-PCR in MCF7 cells/normal breast epithelial cells (MCF10A). CLN3p subcellular localization was determined by immunocytochemistry in MCF10A/MCF7 cells. CLN3p expression was blocked by transfecting MCF7 cells with scrambled siRNA/siRNA directed against *CLN3*. *CLN3* expression impact on cancer cell growth/apoptosis/ceramide production was determined using trypan blue dye exclusion/propidium iodide staining and DGK assay. Effect of different chemotherapeutic agents (Fenretinide: activator of ceramide generation; sodium butyrate: modifier of histone acetylation; 5-aza-2'-deoxycytidine: alters methylation status) on cancer cell growth/apoptosis was determined. *CLN3* expresssion was determined from RNA extracted from fresh/FFPE breast tissue and analyzed by RT-PCR.

Results

CLN3 mRNA is overexpressed in MCF7/MCF10A cells. MCF7 cells overexpressing *CLN*3 show co-localization of CLN3p with Golgi reassembly and stacking protein (GRASP65)/plasma membrane (GAP43)/early recycling endosomes (Rab4)/endoplasmic reticulum (Calreticulin)/late endosomes (Rab7)/lysosomes (Cathepsin D). Blocking *CLN3* expression by siRNA or fenretinide/sodium butyrate/5-aza-2'-deoxycytidine inhibited growth/viability of MCF7 cells, increased apoptosis and pro-apoptotic ceramide. Finally, *CLN3* mRNA is overexpressed in tumor compared to normal breast tissue from fresh samples.

Conclusion

Targeting *CLN3* overexpression provides a novel molecular target for breast cancer drug discovery, possibly acting via modulation of ceramide pathways.

Keywords: *CLN3*, CLN3p, breast cancer, MCF7/MCF10A cells, breast tissue, cell growth, apoptosis, ceramide

ABSTRACT #25

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION AND ITS CLINICAL IMPLEMENTATION: THE AUBMC EXPERIENCE IN NEURODEVELOPMENTAL DISORDERS

Nadine J. Makhoul[1], Simona Ghanem[1], Chantal Farra[2,3], Rose-Mary Boustany[1,3]

[1]Department of Biochemistry and Molecular Genetics, [2]Department of Pathology and Laboratory Medicine, [3]Neurogenetics Program and Division of Pediatric Neurology, Department of Pediatrics & Adolescent Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Corresponding author: Nadine Makhoul - Research Assistant

*E-mail: nm36@aub.edu.lb

Introduction

Array-based comparative genomic hybridization (aCGH) has become a powerful tool for detection of structural chromosomal abnormalities in human diseases. Because of its numerous advantages over conventional cytogenetic diagnostic methods, aCGH is now recommended as a first-tier test for postnatal investigation of individuals with unexplained developmental and intellectual disabilities and/or multiple congenital anomalies. Fast and accurate, high-resolution genomic arrays facilitate the detection of DNA copy number variants (CNV) and allow investigation of their integral genes. Here we present our experience in implementing this technology in 47 patients at AUBMC between September 2010 and November 2012.

Methods

Copy number changes were analyzed in 47 unrelated children referred for developmental delay, mental retardation, dysmorphic features and/or multiple congenital anomalies, using the Affymetrix® Cytogenetics 2.7M arrays. Data was analyzed using ChAS Software and CNVs greater than 500Kb for gains and 200Kb for losses (50Kb in haploinsufficient regions) were classified into 3 categories: likely benign, pathogenic and variants of unknown significance (VUS).

Results

117 CNVs were detected in 43 patients, the remaining 4 patients displaying a normal aCGH, making the average number of CNVs/individual to ~2.5. The most frequent CNVs were duplications (78/117) affecting chromosomes 2 (14%), X (10%), 7 (9%), and 15 (9%), and deletions in chromosomes 14 (25%), 15 (10%), 17 (7%) and X (7%). Six CNVs were likely pathogenic with an average size of 7 Mb (range 126 Kb-15.6 Mb) involving 9q21.33-31.1, 9q33.3-34.3, 17p11.2-12, 17p13.1-13.3, 18q22.2-q23 and Xp21.2-21.3, while seventy-nine (67.5% of total CNVs) were benign, and thirty-two CNVs classified as VUS.

Conclusion

This study agrees with previously reported >10% diagnostic yields of clinically significant CNVs in patients with unexplained mental and developmental disabilities. aCGH has led to better diagnosis by identification of more CNVs in this population offering a higher diagnostic yield than the 1-3% reported for routine karyotype and FISH.

Keywords: array comparative genomic hybridization, copy number variants, intellectual disabilities

ABSTRACT #26

AUTISM SUSCEPTIBILITY GENES IN THE LEBANESE POPULATION

Soueid J., Kourtian S., Makhoul N., Ghanem S., Boustany R-M.

Department of Biochemistry and Molecular Genetics,
Neurogenetics Program and Division of Pediatric Neurology, Department of Pediatrics & Adolescent Medicine,
Corresponding author: Jihane Soueid - postdoctoral fellow
*****E-mail: js61@aub.edu.lb

Introduction
Autism is a neurodevelopmental disorder characterized by ritualistic-repetitive behaviors, impaired non-verbal communication and language development. Prevalence estimates of autism have increased over the last 20 years. Although autism is considered to be genetic in 20-25% of cases, its extreme heterogeneity has defied genetic classification. Few recurrent aberrations include 15q11.2-13 duplication and single gene disorders (Tuberous Sclerosis/Fragile X syndrome/Rett syndrome). Identification of a large number of autism susceptibility genes (*SHANK3*/*NLGN3*/*NLGN4*/*NRXN1)* led to increased appreciation of the contribution of de novo and inherited copy number variation (CNV). Aims are to confirm previously identified autism susceptibility genes in lebanese autism cases and/or uncover novel autism susceptibility genes specific to the Lebanese.

Methods
Cytogenetics 2.7M Microarray technology was used to detect CNVs within/across families of ASD children, and map homozygous regions unique to ASD patients in each family. The Lebanese population is ideal for homozygosity mapping because of shared ancestry and increased siblings per family.

Results
Homozygosity mapping revealed previously described autism/schizophrenia/mental disorder susceptibility genes (CNTNAP2/GLO1/NRXN1/GRIK2). Interestingly, we uncovered novel groups of candidate genes involved in glutamatergic transmission (GABRA1/GRIA1/ALDH9A1) neuronal cell adhesion (NLGN3/CNTN3) and in synapse formation (PTPRT/GOLSYN). Comparing genomes of 28 autistic children narrowed the analysis to common homozygous regions found in ≥6 children. Within these regions, we selected 2 previously described genes: NRXN1 and CNTNAP2, ahead of the three novel candidate ASD susceptibility genes (CA13/THRH/ENY2) to perform mutational screening. We discovered an undescribed heterozygous frameshift mutation in the CNTNAP2 gene in five/seven patients screened that could increase susceptibility for development of ASDs. Also, among CNVs numerous *de novo*/inherited events were identified implicating novel ASD susceptibility genes (DISC1/GRID1/ELOVL7).

Conclusion
Our results identify new genetic/functional targets in ASD. Uncovering a set of genes responsible for autism in the Lebanese population will facilitate diagnostic work-up and genetic counseling for ASD in Lebanon. Discovery of novel ASD genes may lead to untapped and novel therapeutic targets.

Keywords: Autism, Microarray technology, Homozygosity mapping, Copy Number Variations.

ABSTRACT #27

ROAD SIDE LEVELS OF REACTIVE OXYGEN SPECIES

M. Jaafar, A. L. Shihadeh and N. A. Saliba*

Chemistry Department, American University of Beirut, P.O. Box 11-0236, Riad El-Solh, Beirut, 1107 2020, Lebanon
Presenter: Malek Jaafar (Graduate Student)
*Corresponding Author: e-mail address: ns30@aub.edu.lb.

Introduction

Particulate matters (PM) originate from both anthropogenic and biogenic sources. They differ in sizes and can range between a few nanometers and several micrometers in diameter. These PMs carry several forms of mineral oxides, soot, organic and inorganic species of different sources. Of these species we are interested in Reactive oxygen species (ROS) as well as those that form these ROSs. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and/or nitrogen. Within normal levels, they are harmless; the body for example produces ROS which play a role in cellular functions. Yet elevated levels of ROS can cause damage to cellular structures. This study aims to find the ROS levels in PM sampled on Roadside Jal El Dib and AUB which is used as a background.

Method

PM samples (sizes 10 micrometers and below in diameter) are collected on quartz and pre-weighed Teflon filters using Personal Cascade Impactor Samplers (PCIS) connected to a PM10 Harvard Cartridge. The quartz filters were placed in the fridge for other analyses as for the Teflon filters, they were then, conditioned and post-weighed before being analyzed for their total ROS using the DTT assay and UV spectrometer.

Results

ROS values were found to be high, especially in PM sizes below 0.25 micrometers in diameter, which are mainly due to vehicle emissions. The chemicals responsible for these ROS included different classes of compounds, ranging from polyaromatic hydrocarbons (PAH) to metal ions like iron, zinc and vanadium all of which are mainly emitted from vehicle wear. The levels PAHs and heavy metal were already found to be in high based on previous studies.

Conclusion

Although this study is still in its initial steps, the results are still of concern. High ROS values have been attributed to many health issues. Our future plan of work includes expanding this method to sample at different locations throughout Lebanon.

Keywords*:* Traffic Pollution, Reactive Oxygen Species, DTT Assay, Particulate Matter

ABSTRACT #28

DEMONSTRATING THE ANTI-LEUKEMIC EFFECT OF COMBINING ARSENIC TRIOXIDE AND INTERFERON ALPHA ON CML CELL LINES SENSITIVE AND RESISTANT TO IMATINIB

M. Mouteirik [1], A. Iskandarani [1] and R. Nasr [1]

[1]*Department of Anatomy, Cell Biology and Physiological Sciences*
*Emails: -Corresponding Author: Dr. Rihab Nasr, rn03@aub.edu.lb
- MS Graduate Student: Maha Mouteirik, mnm15@aub.edu.lb

Background and Objectives
Chronic myeloid leukemia (CML) is a clonal myeloproliferative stem cell disorder that results from a reciprocal translocation between chromosomes 9 and 22. This translocation moves the *abl* gene next to the *bcr* gene creating the hybrid gene (*bcr-abl)* which codes for a constitutively active tyrosine kinase (BCR-ABL). The result is an expansion in the myeloid series due to an alteration in the balance between cell proliferation and apoptosis. Modern therapeutic approaches basically involve the utilization of tyrosine kinase inhibitors (TKIs) to target the BCR-ABL tyrosine kinase and some of its downstream effectors. Despite the relative safety and feasibility of using TKIs, several obstacles have emerged including drug resistance, intolerance to therapy and economic burden of using new patented drugs. Most importantly, these drugs have succeeded in significantly prolonging the life of CML patients and improving the quality of their life, but TKIs do not provide a cure since patients relapse as soon as they discontinue treatment. Several studies have proved that the reason why patients continue to have the disease despite therapy is the presence of quiescent TKI-insensitive leukemic stem cells (LSCs) within the bone marrow that provide an endless reservoir of leukemic cells. Therefore, the best way to cure CML is to target LSCs especially for patients that respond suboptimally to current treatment with TKIs. Arsenic trioxide is an ancient remedy that was used to treat CML patients with limited success in the mid 1800's; it has recently gained attention in the treatment of hematologic disorders including acute promyelocytic leukemia (APL). Interferon alpha (IFN-α) is another drug that was formerly used to treat CML patients prior to the development of TKIs. Both arsenic and IFN-α have been shown separately to be effective against CML cells in culture and in vivo. The aim of our work is to demonstrate the anti-leukemic effect of combining arsenic and IFN-α on the CML cell line AR230 and its imatinib-resistant counterpart (AR230-R).

Design and Methods
AR230 sensitive and AR230 resistant to imatinib cells were treated with interferon α alone, arsenic alone or a combination of arsenic/interferon. Cell viability was determined using Trypan blue exclusion assay. The effect on cell growth was determined by MTT assay. Cell cycle analysis was performed by propidium iodide staining, and apoptosis was determined by measurement of pre-G0/G1 DNA content and by the TUNEL assay. Also, the effect of treatment on BCR-ABL level and activity will be evaluated by western blot.

Preliminary Results
CML cell lines were treated with clinically achievable concentrations of IFN-α and arsenic. In proliferation analysis, arsenic alone had minimal effect on the tested cell lines whereas interferon alone had a more significant effect. Interestingly, the addition of arsenic to interferon significantly enhanced the inhibition of in AR230 cells. The treatment of cells with arsenic and

IFN showed minimal variation in cell cycle distribution although there was a significant increase in the percentage of cells in the pre-Go stage that are presumably apoptotic cells. The percentage of cells in the pre-G0 stage also increased in the presence of arsenic or IFN alone but to a lesser extent. Collectively, these results indicate that arsenic/ IFN inhibits the growth of CML cell lines mainly through induction of apoptosis.

Conclusion
Arsenic/IFN inhibited the growth and induced apoptosis of CML cell lines. More importantly, arsenic/IFN had anti-leukemic effects on imatinib-resistant cells. Ultimately, the use of this combination should be explored as a curative approach to cure CML patients especially those resistant to currently available TKIs. Ultimately, this study aims to provide a curative approach to treat CML patients including those that present with resistance to currently available TKIs.

Keywords: Chronic myeloid leukemia, BCR-ABL, arsenic trioxide, interferon alpha.

ABSTRACT #29

PROFILING OF MICRO RNA IN LEBANESE BREAST CANCER PATIENTS

El- Sabban, Maya and Nasr, Rihab
Department of Anatomy, Cell Biology and Physiology, American University of Beirut, Lebanon
Participant: Maya El- Sabban: mme65@aub.edu.lb, Second year Masters student
*Email: Rihab Nasr: rn03@aub.edu.lb

Introduction

Breast cancer is the most common cancer among women in both western populations and in Lebanon. However, Lebanese women have an average age of diagnosis that is significantly younger than the western counterpart. This earlier onset of breast cancer is often correlated with a poor prognosis and overall survival. However, at present essentially nothing is known regarding these characteristics. It is therefore crucial to develop a minimally invasive biomarker screening mechanism through the employment of signature biomarkers for the detection of this early onset breast cancer.

microRNAs have been emerging as potential candidates for cancer biomarkers in recent years. miRNA profiles have been characterized in a multitude of cancers not only in the tumor site but circulating in blood, thereby providing the advantage of a non-invasive method of profiling. The aim of this study is to evaluate the possible correlation between miRNA and different clinical parameters, with the hope of identifying miRNA expression patterns predicting prognosis and explaining the peculiarities of breast cancer in Lebanon. Identifying the patterns of miRNA expression in Lebanese patients with early onset breast cancer will eventually translate to not only a global understanding of the disease but also to a diagnostic and prognostic biomarker at our disposal.

Methods

10-20 µm sections that contain 90% tumor tissues were obtained from formalin fixed paraffin-embedded specimens from a cohort of 200 Lebanese breast cancer patients. To study the expression of miR-10b, miR-21, miR-148 and miR-221, total RNA was are being extracted using the RecoverAll kit, cDNA synthesis using looped miRNA-specific reverse transcription primers and qRT-PCR using specific microRNA TaqMan probes are being performed. Furthermore, in order to look at overall microRNA expression patterns, miRNA expression profiling will be conducted using GeneChip miRNA arrays on an Affymetrix platform.

Expected Results

It is expected that the patients who have early onset breast cancer below the age of 35 will display a unique pattern of microRNA dysregulation. This pattern will correlate with the menopausal status, age and receptor status of the patient. It is expected that mir-221 and mir-21 over expression will be correlated with a poorer prognosis and a later cancer stage, and that mir-148 over expression will be correlated with a younger age at diagnosis. These hypotheses have yet to be confirmed however.

Conclusion

It is important to establish whether these potential miRNA patterns exist and whether they will be useful in detecting early onset breast cancer in Lebanese patients. If these patterns are identified from breast tissue samples, then it will be a worthwhile stepping-stone in order to use blood samples as a method of non-invasive screening.

Keywords: miRNA. Biomarker. Breast Cancer.

ABSTRACT #30

PARTICULATE TRAFFIC EMISSIONS IN BEIRUT

R. Baalbaki[a], M. Katurji[b], Q. Al-Assaad[a], C-J. Mhanna[a], M. Roumié[c], and N.A. Saliba[a*]

[a]Chemistry Department, American University of Beirut, P.O. Box 11-0236, Riad El-Solh, Beirut, 1107 2020, Lebanon
[b]Center for Atmospheric Research, University of Canterbury, Christchurch, New Zealand
[c] Accelerator Laboratory, Lebanese Atomic Energy Commission, National Council for Scientific Research, P.O. Box 11-8281, Beirut, Lebanon
Presenter: Rima Baalbaki (Research Assistant)
* Email: Corresponding Author: e-mail address: ns30@aub.edu.lb.

Introduction
Vehicles are a major source of particulate matter (PM) both from direct emission via combustion and mechanical wear, and secondary aerosol formation from emitted gaseous precursors. Exposure to high levels of inhalable PM is associated with adverse short term and chronic health effects such as increased prevalence of respiratory symptoms, decrements in lung function, increased susceptibility to infection, and reduction in life expectancy owing mainly to cardiopulmonary mortality. This study attempts to evaluate real-world vehicle emissions of $PM_{2.5}$ (particles with aerodynamic diameter less than 2.5 μm) on busy roads in - and leading- to Beirut by calculating fuel based emission factors (EFs). Additionally, PM number concentrations on roads are compared to a "background" site; AUB.

Methods
$PM_{2.5}$ samples were collected on polycarbonate filters using Harvard cartridges and the respective $PM_{2.5}$ mass concentrations were determined using a micro-analytical balance. Elemental analysis on the filters was done using the conventional in vacuum PIXE (Proton Induced X-ray Emission). CO_2 concentrations were measured using CO2Meter-K-30 Probe. Particle number concentrations were measured using the GRIMM EDM 365 dust monitor.

Results
Calculated $PM_{2.5}$ mass EFs conform to those of heavy duty vehicles. When compared to roads in California, USA, higher $PM_{2.5}$ mass EFs were obtained suggesting a higher loading in large size particles. Total particle counts measured on the road-side are up to 67% higher than particle counts measured at the background site. In hot, dry and humid summer weather, particle dispersion is shown to be a function the boundary layer thickness with particle counts measured during the morning being around 40% higher than particle counts measured during the afternoon.

Conclusion
Similar to other developing countries, Beirut exhibits high EFs and prevalence of large size particles on road sites. Results are crucial for further health studies and policy reforms.

Keywords: Traffic, Air Pollution, Ambient Air, Emissions, Automotive, Particulate Matter

ABSTRACT #31

SEA CUCUMBER (HOLOTHURIA POLII) EXTRACTS ENHANCE CELL-CELL COMMUNICATION AND DECREASE CELL PROLIFERAION OF HUMAN BREAST AND COLON CANCER CELLS

Sara Al-Ghadban[1], Rabih Talhouk[2], and Marwan El-Sabban[1]

[1]Department of Anatomy, Cell Biology, and Physiological sciences, American University of Beirut, Lebanon
[2]Department of Biology, American University of Beirut, Lebanon
Participant: Sara Al-Ghadban (Research Assistant); sa99@aub.edu.lb
*Email: Corresponding Author: Marwan El-Sabban; me00@aub.edu.lb

Introduction
The unexplored, rich biodiversity of the Mediterranean Sea and marine life has recently attracted the interest of researchers seeking natural bioactive compounds.
The objective of MAREX is to isolate and characterize anti-tumor and anti-inflammatory bioactive compounds from extracts of Lebanese coast sea cucumbers (*Holothuria polii*) (SCE).

Methods
Aqueous ethanolic extracts from ***Holothuria Polii*** were analyzed for their biological characteristics and assessed for various biological constituents. Cell toxicity, proliferation, invasion and migration assays were studied in two cancer cells (breast MDA-231 and colon Caco 2). In addition, tumor cell/endothelial cell interaction and cell-cell communication were investigated in both human breast and intestinal cancer cells.

Results
SCE treatment decreased cancer cell proliferation in a dose-dependent manner with no significant cytotoxicity. The expression of cell-to-cell communication protein (Cx26) in cancer cells was increased as was adhesion and gap-junction intercellular communication in cancer cells upon treatment with SCE. The mechanism by which these extracts exert their action is investigated and the isolation of active components using bio-guided fractionation is under way.

Conclusion
SCE decrease cell proliferation and up-regulate gap-junction intercellular communication (GJIC) *in vitro,* suggesting a potential role of sea cucumber in the development of anti-cancer drugs.

Keywords: Sea cucumber extract; proliferation; invasion; angiogenesis; GJIC

ABSTRACT #32

EXOSOMES: VECTORS FOR BIO-DELIVERY AND A NOVEL "INDUCED CELL-TO-CELL COMMUNICATION" MECHANISM

Jamal El Saghir[1] and Marwan El-Sabban[1]

[1]Department of Anatomy, Cell Biology, and Physiology, Faculty of Medicine, American University of Beirut, Lebanon
Participant: Jamal El Saghir, je08@aub.edu.lb (Research Assistant)
*Email: Corresponding Author: Dr. Marwan El-Sabban, me00@aub.edu.lb

Background and aims
Exosomes are membrane nano-vesicles secreted by a multitude of cells under both physiological and pathological conditions. They were identified in most body fluids such as blood, milk, urine and saliva. Although initially exosomes were thought to export cell debris, they were found to contain biologic information such as protein, mRNA and miRNA that can be transferred to other cells, implicating them in many patho-physiological processes. In this study, two approaches were adopted to understand the role of exosomes in intercellular communication. On one hand, we investigated if connexins (the gap junction proteins)-associated with exosomes, transfer to recipient cells, and investigate whether incorporation of these connexins into the new cells alter their functionality. In addition, we examined if Tax (viral oncoprotein)-containing exosomes from HTLV-I (human T-cell lymphotrophic virus type I) infected cells adhere and subsequently deliver Tax to recipient cells.

Methods
293T cells were transfected with Cx26- or Cx43-Dendra -fluorescent protein. 48h post-transfection, exosomes were purified from culture supernatants by differential ultra-centrifugation and examined either by RT-PCR to detect Cx26-Dendra transcripts or by western blotting to detect the exosomal marker CD63 and Cx26-/Cx43-Dendra proteins. In addition, HuT-102 cells were cultured in serum-deprived media for 72h and exosomes were purified from culture supernatants and examined by western blotting for CD63 and Tax expression. Finally, we investigated Tax expression in endothelial cells following co-culture with HuT-102 exosomes for 24h.

Results
Cx26-Dendra transcript and Cx26-/Cx43-Dendra proteins were detected in the exosomes of 293T cells transfected with Cx26D and not in those of un-transfected 293T cells. We also showed that Tax-containing exosomes from HTLV-I cells adhere and deliver Tax to recipient cells.

Conclusion
These preliminary findings demonstrate that exosomes cargo may contain connexins and Tax oncoprotein. Further experiments will test the functionality of these specific cargo proteins on recipient cells.

Keywords: exosomes, intercellular communication, connexins, HTLV-1.

ABSTRACT #33

CONTROL OF A TELE-HAPTIC PNEUMATIC ACTUATOR FOR MRI APPLICATIONS

Aly Elbadry Elmasry[1], Prof. Mathias Liermann[2]

[1]Undergraduate research assistant, [2]Research supervisor
Email: ali.elbadry@gmail.com
matthias.liermann@aub.edu.lb

Background and aim

The usage of control and tele-operation in the field of engineering is numerous. One of the usages is MRI-guided surgery. Pneumatic actuators are often used in MRI to avoid the complications that come with electromagnetic actuators. In addition to using pneumatic actuation there is often a need for haptic feedback, so the manipulator feels what is happening on the other end. The aim of this research project is to set up a prototype for tele-haptic bio-medical robot.

Method

The poster presents an experimental setup on which we plan to implement various control strategies for tele-haptic manipulation under MRI guidance. The robot could consist of several axes, of which one may have the structure as shown in the figure below

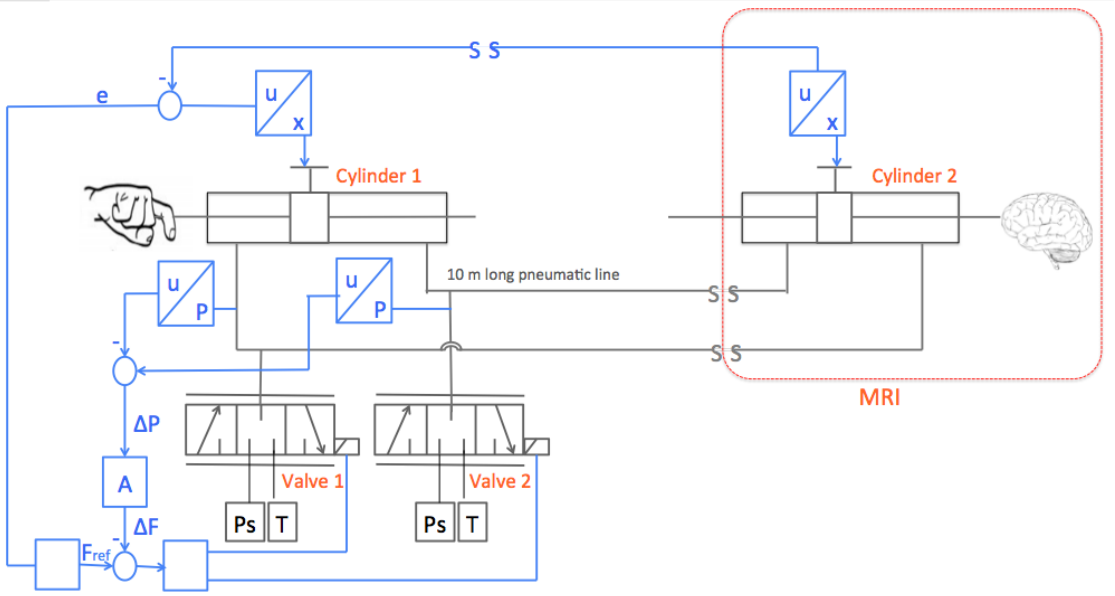


FIGURE 1: SCHEMATIC FIGURE FOR TELE-HAPTIC PNEUMATIC ACTUATOR

Two low-friction cylinders are connected directly with each other. Cylinder 1 would be operated by hand; Cylinder 2 would be inside an MRI machine in an adjacent room. Thus our manual input would be the operator's hand on cylinder 1. The pneumatic lines are long enough to connect both actuators (10 m). Two pressure sensors will be connected to both pneumatic lines connecting the cylinders, and the difference in pressures will be calculated. From that we will be able to extract the difference in forces and correct for the force reference. Two position sensors will be able to calculate the difference between the manual movement of cylinder 1 and the

controlled movement of cylinder 2. The valves are controlled in order to set the passive stiffness of the setup or to simulate desired impedance given the input from the sensors.

Results and Conclusion

The experimental setup is shown in the figure below; a simple control scheme was used that resulted in an immediate response of the remote axis. The operator could sense the forces acting on the remote actuator. We are continuing to implement more sophisticated control schemes and to quantify the influence of friction in the cylinders on the force feedback accuracy.

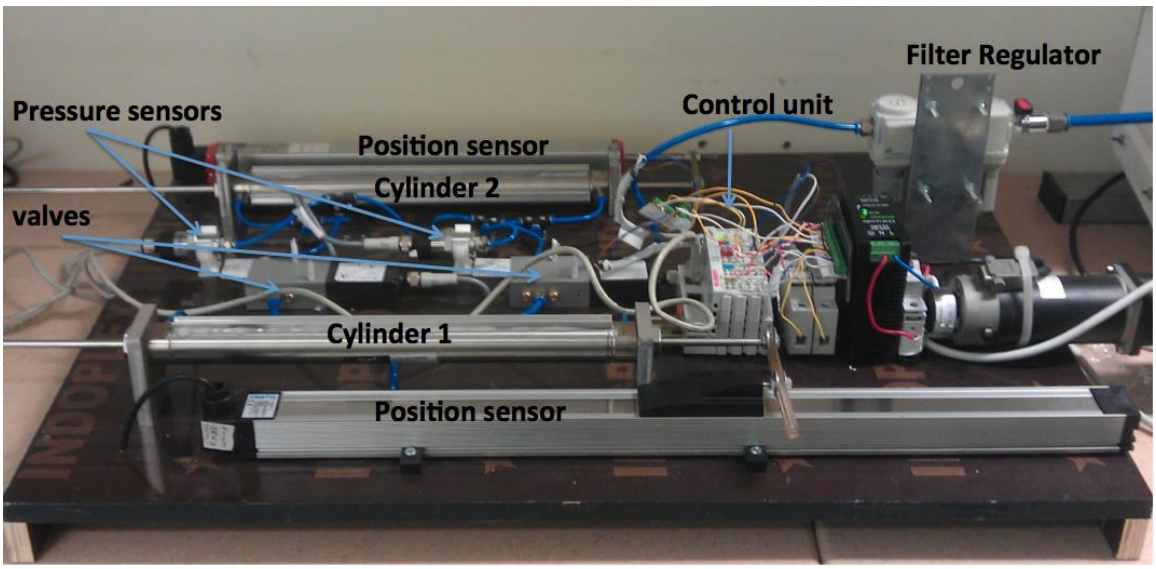


FIGURE 2: PHYSICAL SETUP FOR TELE-HAPTIC PNEUMATIC ACTUATOR

Keywords: Tele-haptic actuator, MRI guided surgery, force feedback

ABSTRACT #34

THE EFFECT OF REACTIVE OXYGEN SPECIES GENERATION ON THE CROSSTALK BETWEEN BRADYKININ AND SPHINGOSINE-1-PHOSPHATE RECEPTORS IN VASCULAR SMOOTH MUSCLE CELLS

Kazezian Z#, Al Hariri M#, Abou Msallem J#, Eid A*, Ziyadeh F#, Jaffa A#§.

[1]Department of Biochemistry and Molecular Genetics. [2]Department of Internal Medicine,
[3]Department of Anatomy, Cell Biology and Physiological Sciences, Faculty of Medicine.
American University of Beirut.
Presenter: Research assistant
*Email: joe.aboumsallem@gmail.com

Background
Atherosclerosis is a silent chronic inflammatory disease that is a major cause of death worldwide. Due to the injury of endothelial cells aligning the aorta, the blood contents such as white blood cells, cytokines, growth factors and lipids interact directly with the smooth muscle cells of the vessel wall leading to vascular remodeling and lesion formation. Reactive oxygen species (ROS) generation in vascular smooth muscle cells activates signaling pathways, which contribute to vascular remodeling.

Hypothesis
We hypothesized that kallikrein-kinin system (KKS) especially bradykinin (BK) has a role in vascular remodeling via ROS generation, crosstalk with Sphingosine-1-Phosphate receptors (S1PR)s, and inducing the signaling pathways.

Results
When BK acts directly on the smooth muscle cells leading to the synthesis of ROS; ROS in turn activate signaling pathways like mitogen-activated protein kinases (MAPK)s and Phosphatidylinositol-3 kinase (PI3K). This was confirmed by using Nacetylcysteine (NAC), a scavenger of ROS that led to a significant decrease in extracellular signal-regulated kinase (ERK *1/2)* and AKT. We also discovered that there is a crosstalk between bradykinin and S1PRs via sphingosine kinase 1 (SphK1) due to ROS generation by BK. On the other hand, when we used NAC, the ROS generation and the downstream signaling pathways of bradykinin 2 receptor (B2R) were inhibited, also CTFG, Fibrtonectin and (SphK1) gene expression was decreased. Besides, we verified that S1P activates MAPK and PI3K pathways by increasing ERK 1/2 and AKT respectively, which leads to proliferation and migration of smooth muscle cells. SIP also increases CTGF and Fibronectin production, which leads to the production of extracellular matrix (ECM) particularly by S1PR1

Conclusion
The results suggest that BK induced ROS generation activates ERK 1*12* and AKT to promote vascular remodeling. Moreover, BK initiates a crosstalk between B2R and S1PRs (EDGRs) via activation of SphK1. On the other hand, SIP plays a role in vascular remodeling by activation of MAPK and PI3K pathways and also increases CTGF and Fibronectin production.

Keywords: Atherosclerosis, cell signaling, Vascular Remodeling, Bradykinin, Reactive Oxygen Species.

ABSTRACT #35

ASSESSMENT OF ORAL HEALTH IN ELEMENTARY SCHOOL CHILDREN IN BEIRUT: A COMPARISON BETWEEN PRIVATE AND PUBLIC SCHOOLS

Antoine E. HANNA, Celine M. MOUKARZEL, Ramzi V. HADDAD, Monique CHAAYA, Miran JAFFA, Joseph G. GHAFARI

Dr. Antoine Hanna (Post-Doctoral Fellow), Dr. Celine Moukarzel: Former residents, Division of Orthodontics and Dentofacial Orthopedics, Department of Otolaryngology Head and Neck Surgery, AUBMC; former MS students, Department of Epidemiology and Population Health, FHS.

This abstract combines the separate research theses of Dr.'s Hanna and Moukarzel toward their MS degrees in Epidemiology

RESEARCH ADVISORS AND CONTRIBUTORS

Dr. Miran Jaffa, Dr. Monique Chaaya: Department of Epidemiology and Population Health.

Dr. Ramzi V. Haddad, Dr. Joseph Ghafari: Division of Orthodontics and Dentofacial Orthopedics, Department of Otolaryngology.

*Email: Dr. Antoine Hanna: ah111@aub.edu.lb (X5700).

Oral health encompassing dental decays, oral hygiene and malocclusion (bite problems) varies with socioeconomic and educational backgrounds.

Aims

1- Compare indices of various components of oral health between children in private (PV) and public (PB) schools in Lebanon.

2- Investigate associated demographic, socioeconomic, and behavioral factors.

Methods

Malocclusion, DMFT (Decayed, Missing, Filled Teeth) and plaque (hygiene gauge) indices were measured in 655 elementary school children (6-11 years) in PB (n=325; 153 girls, 172 boys) and PV (n=330; 177 girls, 153 boys) in Beirut. Calibrated dentists recorded: 1- occlusal parameters: overjet (OJ), overbite (OB), posterior crossbite (PXB), midline diastema and crowding (Irregularity Index), 2- DMFT score and 3- Plaque index. Following standardized procedures. Socio-demographic and behavioral data regarding children and parents were collected through a questionnaire completed by the parents.

Results

The mean DMFT score was statistically significantly higher (7.50±3.98) in PB compared to PV (3.50±3.41). The mean plaque index was also greater in PB (1.35±0.23) compared to PV (1.20±0.15) ($p<0.001$). A higher DMFT score was associated with poor oral health perception, breast feeding and public school. Plaque index was associated with the oral health perception. Malocclusion in its major components (OJ, anterior XB, and occlusal relations) was statistically significantly more severe in PB versus PV. Age was positively associated with OJ, OB and PXB. Increased sucking habit duration was associated with a shallower OB and PXB. Crowding was more severe among males and associated with an increase in the DMFT score. Computed orthodontic treatment need scores revealed that nearly 25% of the children are in urgent need of treatment.

Conclusion

DMFT and malocclusion severity scores were higher in public than private schools, and were higher than similar data in the USA and European countries. Accordingly, more prevention strategies are needed in Lebanon with more attention to children in public schools.

Keywords: Oral health, DMFT score, Plaque index, Malocclusion, Public, Private, Beirut.

ABSTRACT #36

CORRELATION BETWEEN VOICE PARAMETERS AND CRANIOFACIAL MORPHOLOGIES IN PAEDIATRIC AND ADULT POPULATIONS

Macari Anthony [1], Karam Ingrid [1], Tabri Dollen [2], Hamdan Abdul Latif [2]
Division of Orthodontics and Dentofacial Orthopedics, Department of Otolaryngology Head and Neck Surgery, American University of Beirut Medical Center
Division of Laryngology and Hamdan Voice Unit, Department of Otolaryngology Head and Neck Surgery, American University of Beirut Medical Center
Presenter: Ingrid Karam, DDS: Resident, PGY3, Division of Orthodontics and Dentofacial Orthopedics, Department of Otolaryngology Head and Neck Surgery, American University of Beirut Medical Center.
Advisor: Dr. Anthony Macari
*Email: ik27@aub.edu.lb

Introduction
Patients are able to adapt their speech to their facial deformities and malocclusions (bite problems). Speech disorders have been reported with malocclusions such as Class III (reverse bite, mandibular prognathism), but the association between facial and dental components and voice needs investigation.

Aim To study the correlation between voice parameters and craniofacial morphology.

Methods
A preliminary pool of 40 patients (age:9-40 years) seeking orthodontic treatment underwent a standard voice test to analyze a number of parameters including fundamental frequency (F0) and habitual pitch (HP). Craniofacial morphology was assessed on lateral cephalometric radiographs. The following cephalometric measurements were computed: maxillary length (anterior to posterior nasal spine; ANS-PNS in mm); mandibular length (from condyle to anterior part of the chin; Co-Gn in mm); inter-jaws relationship in the sagittal (ANB angle connecting the mandible and the maxilla to Nasion at the fronto-nasal intersection), and vertical (angle between palatal and mandibular planes; PP/MP) dimensions. Statistical analyses included t-tests for group differences and the Pearson's product moment correlation for associations among variables.

Results
No statistically significant differences ($p>0.05$) were found for any voice variable between patients with normal Class I (n=19) and Class II (increased horizontal overbite; n=18) malocclusions. All patients with Class III (n=3), nearly half of those with Class II, and only 11% with Class I had F0 and HP in the highest range (>230). Moderate negative correlations were observed between mandibular length and F0 ($r=-0.54$) and HP ($r=-0.56$). Low correlations ($r<0.35$) were found between voice parameters and the sagittal (ANB) and vertical (PP/MP) jaw relations. F0 and HP decreased with age.

Conclusion
Moderate correlations found between voice characteristics and mandibular size but not with intermaxillary relations indicate more involvement of the mandible in voice definition, The disparity between malocclusions warrants further investigation with a larger sample including more patients with Class III.
Key words: craniofacial morphology, voice parameters, malocclusion.

ABSTRACT #37

SUB-LETHAL HIGH INTENSITY FOCUSED ULTRASOUND EXPOSURE RESULTS IN ALTERED MECHANOSENSITIVE GENE EXPRESSION IN MAMMARY EPITHELIAL CELLS

Lori H. Malkhassian[1], Moath Al Qraini[2], Hind Zahr[1], Ghanem Oweis[2], and Diana E. Jaalouk[1*]

[1]Department of Biology, [2]Department of Mechanical Engineering, American University of Beirut, Beirut, Lebanon.

Email: dj11@aub.edu.lb

Background & Aims

High Intensity Focused Ultrasound (HIFU) is a non-invasive therapeutic modality that allows the ablation of unwanted tissues inside the body. In recent years, HIFU gained much attention for its potential applications in the treatment of multiple disorders including breast cancer. At the focal spot, intensified energy results in immediate cell death due to instantaneous temperature rise and/or cavitation effects in the target tissue. The surrounding tissue, though routinely receives residual energy, is believed to be left undamaged. Considering that HIFU exposure results in pressure/tension waves which can potentially cause cellular deformations in exposed tissue due to altered mechanotransduction, and that a number of mechanosensitive genes have been shown to be implicated in tumorigenesis, we hypothesize that sub-lethal HIFU treatment would result in mechanotransduction alterations that may induce tumorigenesis of mammary epithelial cells. The objective of this study is to investigate the *in vitro* effects of residual HIFU exposure on cellular viability, proliferation, and mechanosensitive gene expression in mammary epithelial cells.

Methods

An experimental setup was custom-designed and manufactured to permit utilization of a 2.158 MHz HIFU transducer for *in vitro* exposure of MCF-10A immortalized human mammary epithelial cells and MDA-MB-231 invasive human breast cancer cells. Cellular viability and proliferation were assessed days 1-to-4 post HIFU treatment using: i) trypan blue vital stain exclusion assay, ii) WST-1 assay, and iii) standard phase-contrast microscopy. Gene expression was quantified using Real-Time PCR.

Results

Our results show significant decrease in cellular viability and proliferation of MCF-10A and MDA-MB-231 cells exposed at the focal spot in comparison to mock controls ($P<0.01$ for both). Post exposure to 2.5% and 22% residual ultrasound intensity, we find no significant difference in cellular viability and proliferation in comparison to the mock controls in both cell lines. Quantification of *CAV-1α* (encodes for caveolin-1 α) and *PXN* (encodes for paxillin) gene expression in MCF-10A cells shows 1.65- and 2.39- fold increase in *CAV-1α*; 1.54- and 2.40-fold increase in *PXN*, when assessed at 1 hour following 2.5% and 22% HIFU application respectively (mean fold change, $P<0.01$).

Conclusion & Future Directions

Our findings indicate that while a single exposure to residual HIFU has no significant effect on cellular viability and proliferation in MCF-10A and MDA-MB-231 cells, it does result in a significant immediate-early increase in the transcriptional levels of two mechanosensitive genes known to be implicated in mammary tumorigenesis. Future directions will address the bio-functional relevance of these modulations by evaluating their effects on cell motility, migration, invasiveness, and cytotoxic response to anti-neoplatsic agents, hence providing insights into the potential role of sub-lethal HIFU exposure in breast cancer pathogenesis.

Key words: HIFU, mechanotransduction, breast cancer

ABSTRACT #38

BOTH PML NUCLEAR BODIES AND RNF4 ARE REQUIRED FOR ARSENIC/INTERFERON-INDUCED DEGRADATION OF THE HTLV-I ONCOPROTEIN TAX

Zeina Dassouki*[1], Umut shahin*[3], Youmna Kfoury [1], Hiba El Hajj[1], Marwan El-Sabban[2], Kazem Zibara, Rihab nasr[1], Olivier Hermine[4], Hugues de Thé[3] and Ali Bazarbachi[1]

*Equal contribution of DASSOUKI AND SHAHIN
[1]Department of Internal Medicine, and [2]Department of Human Morphology, American University of Beirut;
[3]UMR 7212 CNRS, U944 Inserm, Laboratoire Associé au Comité de Paris de la Ligue contre le Cancer, Paris, France;
[4]CNRS UMR 8603 and Department of Hematology, Necker Hospital, Paris, France;

Adult T cell leukemia (ATL) is one of the rare human cancers initiated by a transforming retrovirus, HTLV-I. After many years of controversy, it is accepted that the viral protein Tax plays a critical role in initiating the leukemic process. We previously showed that the combination of arsenic trioxide (As) and interferon-alpha (IFN□) triggers Tax proteolysis resulting in apoptosis of HTLV-I transformed cells and cure murine ATL derived from Tax transgenics.
However, the biochemical pathways involved in Tax degradation remain unclear. In this study, we show that As/IFN induces the polysumoylation of Tax leading to its subsequent ubiquitylation and proteasomal degradation in both Tax transfected and HTLV-1 transformed cells. Furthermore, this combination fails to degrade Tax when the lysine sites involved in Sumoylation and Ubiquitynation are mutated. Moreover, silencing SUMO-1, 2 and 3 in HeLa cells inhibits Tax degradation. Strikingly, IFN treatment results in complete co-localization of Tax nuclear bodies (NBs) with PML or SUMO2/3 NBs. Furthermore, As/IFN induced Tax SUMOylation then degradation is impaired in PML-Knock-down Hela cells, and delayed in HTLV-1 transformed cells. These results strongly suggest that Tax SUMOylation occurs in PML NBs. To further understand the role of PML in leukemogenesis and Tax proteolysis, we knocked down PML in HTLV-1 transformed cells using short hairpin (shRNA) lentiviral vectors. Subsequently, we inoculated these cells into immunocompromised NOD SCID mice to assess their survival and their organ infiltration. Our preliminary results show that the down regulation of PML in HTLV-I transformed cell lines prolongs mice survival. Finally, we demonstrate that the RING-domain-containing ubiquitin E3 ligase, RNF4, targets poly-SUMO-modified Tax for degradation. This degradation is mediated by Tax ubiquitylation and subsequent recruitment of the 20S proteasome into Tax nuclear bodies. RNF4 depletion leads to the accumulation of poly-SUMO chains and impaired As/IFN-induced Tax ubiquitylation. All together, our results elucidate, for the first time, the molecular mechanism of Tax degradation upon As/IFN treatment.

Keywords: HTLV-I, Tax, PML, RNF4

ABSTRACT #39

MOLECULAR MECHANISMS OF HYPERTENSION-INDUCED LEPTIN EXPRESSION IN VASCULAR SMOOTH MUSCLE

Nadia Soudani[1]; Zein Farrhat[1]; Aida Habib[2]; Ayad Jaffa[2]; Asad Zeidan[1]

[1]Department of Anatomy, Cell Biology and Physiological Sciences, and [2]Department of Biochemistry and Molecular Genetics, Faculty of medicine, American University of Beirut, Lebanon.
*E-mail address of corresponding author: az29@aub.edu.lb

Background
Leptin contributes to several metabolic disorders such as obesity and diabetes mellitus. However, recent studies have linked leptin to cardiovascular pathologies such as vascular smooth muscle remodeling (VSMC), which in turn leads to VSMC remodeling. Mechanisms involving leptin's production and the cross talk between signaling pathways are still not clear. The main aim of this study is to investigate the molecular mechanism that underlies behind mechanical stretch-induced leptin synthesis.

Methods
Organ cultures of rat portal veins (RPV) were either unstretched (control) or stretched (mimic hypertension) by weight at different time intervals (0, 15, 30, 60 min and 24 hr). The effect of atrovastatin (12 mM), actinomycin (a transcriptional inhibitor- 0.1mM), or cycloheximide (a translational inhibitor- 1mM) on mechanical stretch-induced leptin production was studied. Protein expression was assessed using Western blotting and immunohistochemistry methods.

Results
Mechanical stretching of the RPV gradually up-regulated leptin's expression in VSMC to reach its peak after 1 hr of stretching. Both Actinomysin, and cycloheximide significantly inhibited mechanical stretch induced-leptin expression indicating an intervention at a molecular level. Atrovastatin significantly attenuated mechanical stretch-induced leptin expression, ERK1/2 and cofilin phosphorylation. Moreover, the hypertrophic effect of mechanical stretch on VSMC was associated with an increase in calcineurin activation (4 folds).

Conclusion
Our results indicate that the mechanical stretch-induced leptin expression plays a pivotal role in mechanotransduction leading to the development of VSMC hypertrophy. The therapeutic affect the antihypertensive drug "atrovastatin" in attenuating vascular remodeling might be through the inhibition of leptin production, ERK and cofilin phosphorylation.

Keywords: Vascular Smooth Muscle Cells, Hypertension, Hypertrophy, Leptin.

ABSTRACT #40

ESSENTIAL ROLE OF ROS IN MEDIATING STRETCH-INDUCED LEPTIN SECRETION AND VASCULAR SMOOTH MUSCLE HYPERTROPHY

Crystal Ghantous[1]; Leila Dib[1]; Mustafa Al-Hariri[2]; Asad Zeidan[1]

[1] Department of Anatomy, Cell Biology and Physiological Sciences, and [2]Department of Biochemistry and Molecular Genetics, Faculty of medicine, American University of Beirut, Lebanon.
*E-mail address of corresponding author: az29@aub.edu.lb

Background
Obesity is associated with hypertension and increased leptin production that contribute to cardiovascular remodeling. Mechanical stretch (MS) has been shown to contribute to vascular remodeling through various mechanisms, including leptin secretion and vascular smooth muscle (VSMC) hypertrophy.

Methods
We used rat portal vein (RPV) organ culture to investigate the effect of mechanical stretch (mimicking hypertension) on autocrine secretion of leptin and the effect of exogenous leptin (3.1 nM) on VSMC. *Results*: Stretching the RPV for 2, 3 6 or 12h significantly up-regulated leptin gene expressions. In addition, stretching RPV for 24h significantly increased leptin secretion. MS significantly increased ROS production (10 fold increase), effects that was significantly attenuated by the coadministration of an anti-leptin antibody (166 ng/ml), the ROCK inhibitor Y-27632 (10 μM) as well the RhoA inhibitor C3, (30 μg/ml). Disruption of actin microfilaments with 50nM latrunculin B significantly attenuated mechanical stretch-induced ROS production. The role of ROS in MS-induced leptin secretion and expression was further established when pretreatment of NADPH oxidase inhibitor **apocynin (1 mM)** potently attenuated leptin expression and secretion induced by MS. In addition, MS significantly increased polymerization of actin in unstretched blood vessels, as reflected by an increase in the F-/G-actin ratio, effects that were significantly attenuated by **apocynin.**

Conclusion
The hypertrophic effect of mechanical stretch was significantly attenuated by an anti-leptin receptor antibody, and **apocynin**. Our results indicate that the activation RhoA pathway and ROS production plays a pivotal role in MS signaling, leading to leptin secretion and the development of VSMC hypertrophy.

Keywords: Vascular Smooth Muscle Cells, Hypertrophy, Hypertension, Leptin.
Abstract

ABSTRACT #41

CORONARY ARTERY CALCIUM SCORE SIGNIFICANCY ALTERS TREATMENT INDICATIONS IN COMPARISON TO EUROPEAN AND FRAMINGHAM RISK SCORING SYSTEMS AMONG STRESS TEST NEGATIVE PATIENTS

Almedawar M.[1], Harbieh B. [1], Al-Ajaji W. [1], Abchee A. [1], El-Merhi F. [2], Hourani M. [2], Khoury N. [2] , Alam S[1]., Al-Shaar L[1]., Badr K[1]., Isma'eel H[1]*.

Vascular Medicine Program
Departments of Internal Medicine[1] and Radiology[2]
American University of Beirut Medical Center
Beirut, Lebanon
*Corresponding Author: hi09@aub.edu.lb

Background
The Framingham risk score (FRS) is used by the Canadian Guidelines to categorize patients into low (<10%), intermediate (**10%-20%)**, and high (**>20%)** 10-year risk of developing cardiovascular (CV) disease. Similarly, the European Heart Score (EHS) classifies patients into low (<1%), intermediate (1-5%), and high (>5%) 10-year risk for CV death. Moreover, the Coronary Artery Calcium Score (CACS) is an imaging tool that categorizes patients into low (<25th percentile), intermediate (25th -75th percentile) and high (>75th percentile) 10-year CV event rates and has higher predictive accuracy than the preceding 2 scores.

Objective
To determine whether the treatment path chosen for patients based on FRS or EHS would be altered when stratifying the risk with CAC Score and Percentile.

Methods
Data was collected for 138 consecutive patients (55.69 ± 11.70 y.o., 75.4 % Males) who have a negative stress test result for ischemia and have performed CACS. Their FRS and EHS were calculated through predefined formulas. Kappa level of agreement between treatment courses as per FRS, EHS and CACS was tested.

Results
As per CAC Score, 8.1% of the participants not treated according to European Score required lipid lowering therapy with a poor level of agreement (Kappa = 0.195 , p=0.002). Moreover, CACS predicted that 6.6 % not treated according to FRS required drug therapy with a fair level of agreement (Kappa = 0.425, p<0.001). As per CAC Percentile, 37.1% of the participants not treated according to European Score required lipid lowering therapy with a poor level of agreement (Kappa = 0.113 , p=0.172). Moreover, CAC Percentile predicted that 30.8% not treated according to FRS required drug therapy with a slight level of agreement (Kappa = 0.350, p<0.001).

Conclusion
Using CAC Score and CAC percentile will alter the treatment routes and identify people in need of lipid lowering therapy (8.1%- 6.6%) and (37.1%- 30.8%) respectively. Levels of agreement with pathways depending on non-imaging based tools ranged between poor to fair and poor to slight, respectively.

Keywords: Coronary Artery Calcium Score, European Heart Score, Framingham risk score, lipid lowering therapy, Reclassification,

Descriptive statement:
This study found that some patients, who have been found as not requiring lipid lowering therapy as per pathways depending on non-imaging based tools, are actually in need of therapy based on Coronary Artery Calcium Score and Percentile.

ABSTRACT #42

CROSSTALK BETWEEN BRADYKININ AND EGF RECEPTOR AND VASCULAR REMODELING

Saad L[1], Al Hariri M[1], Ziyadeh F[1,2], Eid A.[3] and Jaffa A[1]

[1]Department of Biochemistry and Molecular Genetics. [2]Department of Internal Medicine,
[3]Department of Anatomy, Cell Biology and Physiological Sciences, Faculty of Medicine.
American University of Beirut.
*Email: Lamis Saad, Research Assistant, ls51@aub.edu.lb

Background
Atherosclerosis is the leading cause of death in diabetes, and is a major source of morbidity and mortality. Early atherosclerotic lesions are characterized by endothelial dysfunction, accumulation of inflammatory cells, VSMC proliferation and migration, and extracellular matrix deposition in the vessel wall. Although the association of chronic hyperglycemia and dyslipidemia with diabetic micro-and macrovascular disease is well recognized, the factors and cellular signaling mechanisms that link hyperglycemia and dyslipidemia with atherosclerotic vascular disease are not fully defined.

Aim
Both bradykinin and EGF receptor have been shown to promote vascular remodeling, but the cross-talk between the two systems has not been explored.

Results
Treatment of vascular smooth muscle cells (VSMC) with BK stimulated the mRNA levels of EGFR, MMP2, MMP9 and NOX4. On the other hand treatment of VSMC with EGF induced the mRNA levels of NOX1 and the expression of connective tissue growth factor (CTGF). Both BK and EGF stimulation resulted in the activation of p42/p44 MAPK and AKT. These findings are the first to demonstrate that BK can stimulate EGF expression and its receptor in VSMC through MMPs.

Conclusion
Transactivation of EGF-receptor (EGFR) by G-protein coupled receptors (GPCRs) through Matrix Metalloproteases (MMP) is emerging as an important pathway in cell proliferation, which plays a crucial role in the development of atherosclerotic lesion. Insights into the cellular mechanisms and interrelationships between BK and EGF may provide a novel mechanistic pathway through which both factors interact to promote vascular remodeling.

Keywords: Atherosclerosis, Vascular remodeling, EGF, cell signaling, bradykinin, MMP

ABSTRACT #43

GLOBAL RENAL GENE EXPRESSION PROFILING ANALYSIS IN B2-KININ RECEPTOR NULL MICE: IMPACT OF DIABETES

Jaffa M.[1], Kobeissy F.[2], Al Hariri M.[2], Chalhoub H.[2], Eid A.[3], Ziyadeh F.[2],Jaffa A.[2,4]

*Epidemiology and Population Health Department, Faculty of Health Sciences and #Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Lebanon and §Department of Medicine, Medical University of South Carolina, Charleston, SC, USA
*Email: maa161@aub.edu.lb

Background
Diabetic nephropathy (DN), the leading cause of end-stage renal failure, is clinically manifested by albuminuria and a progressive decline in glomerular filtration rate. The risk factors and mechanisms that contribute to the development and progression of DN are still incompletely defined.

Aim
A genome wide approach to study the effects of diabetes on differential renal gene expression profile in wild type and B_2R knockout ($B_2R^{-/-}$) mice was used to address the involvement of bradykinin B_2-receptors (B_2R) in DN.

Methods
Diabetes was induced with streptozotocin and plasma glucose levels and albumin excretion rate (AER) were measured at predetermined times throughout the 23 week study period. Longitudinal analysis of AER indicated that diabetic $B_2R^{-/-}D$ null mice had a significantly decreased AER levels compared to wild type $B_2R^{+/+}D$ mice (P=0.0005).

Results
Results from the global microarray study comparing gene expression profiles among four groups of mice respectively: ($B_2R^{+/+}C$, $B_2R^{+/+}D$, $B_2R^{-/-}C$ and $B_2R^{-/-}D$) highlighted the role of several altered pathological pathways in response to disruption of B_2R and to the diabetic state that included: endothelial injury, oxidative stress, insulin and lipid metabolism and inflammatory process with a marked alteration in the pro-apoptotic genes.

Conclusion
The findings of the present study provide a global genomics view of biomarkers that highlight the mechanisms and putative pathways involved in DN.

Keywords: Diabetes, Diabetic nephropathy, Bradykinin, Knock-out, and microarray

ABSTRACT #44

EFFECT OF VITAMIN D REPLACEMENT ON IMMUNE FUNCTION AND CONGITION IN MULTIPLE SCLEROSIS (MS)

Hala J Darwish, Raed Ismail, Yasmine Papas, Elie El-Hajj, Nuhad Abou-Zeid, Bassem Yamout and Samia J Khoury

Background
Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system that is linked to genetic and environmental factors such as vitamin D status. Moreover, recent population-based studies linked low serum vitamin D levels to cognitive dysfunction in older adults. New evidence suggests that vitamin D has an immunomodulatory effect in multiple sclerosis. This is a prospective study to evaluate the effect of vitamin D supplementation in MS patients with Vitamin D deficiency (serum level <25µg/ml) to those with normal Vitamin D (serum level >35 µg/ml) on immunological and neuropsychological (cognitive) measures.

Methods
Eighty-six patients diagnosed with relapsing remitting MS or clinically isolated syndrome aged 18 years and older treated with interferon-beta and without signs of active inflammation or cognitive impairment will be recruited. Demographic and health behavior information will be collected, patients will be screened for depression and anxiety using the Arabic- Hopkins Symptoms Checklist (HSCL-25), cognitive performance is measured using the Arabic-Montreal Cognitive Assessment (MoCA) and Stroop Test, Symbol digit Modalities Test (SDMT) and the Brief Visual Memory Test (BVMT). Blood will be collected to examine their vitamin D, calcium and immune profile. Subjects are evaluated at baseline and 3 months after vitamin D supplementation (10,000 IU daily for 3 months or 50,000 IU weekly for 3 months).

Results
Preliminary descriptive behavioral data analysis of the first 20 patients (8 males and 12 females) shows that the mean age is 39.75 (14.57), 52.6 % of them have vitamin D deficiency, 38.3 % and 33. 3% showed mild to moderate depression and anxiety levels respectively, 75 % scored below normal on the MoCA test, 44.4 % showed low resistance on the Stroop test, 72% performed abnormally on the SDMT indicating impaired attention and speed of processing. Spearman Rho test showed statistically significant bivariate correlation between the cognitive tests and between years of education, age and cognitive performance

Conclusion
This preliminary analysis shows a significant proportion of subjects with vitamin D deficiency and cognitive impairment. The significant correlations between the cognitive tests, age and years of education provides a validation of these tests that are being administered for the first time in Arabic on MS patients.

Keywords: Multiple sclerosis, vitamin D deficiency, cognitive functioning, immunomodulation.

ABSTRACT #45

ENHANCEMENT OF ALVEOLAR GAS EXCHANGE BY IMPOSED OSCILLATORY AIT PRESSUE

Ibrahim Chamseddine[1], Issam Lakkis[2], Robert Habib[3], Alan Louis Shihadeh[4]

[1]Corresponding Author, Department of Mechanical Engineering, American University of Beirut
[2]Research Advisor, Department of Mechanical Engineering, American University of Beirut
[3]Research Co-advisor, Department of Internal Medicine, American University of Beirut
[4]Research Co-advisor, Department of Mechanical Engineering, American University of Beirut
Email: imc02@aub.edu.lb

Introduction
It is observed clinically that wideband pressure oscillations enhance oxygenation of blood flow in pulmonary capillaries. One application of this phenomenon is to impose pressure oscillations on the inhaled air to ventilate ill infants with respiratory distress syndrome. The objective of this research is to study the effect of these oscillations on the alveolar gas exchange.

Methods
This study is based on modeling the alveolus and the gas exchange across alveolar membrane. The proposed model that takes as its input a wideband oscillatory pressure signal, predicts the dynamic oxygenation of blood flowing through the pulmonary capillaries while accounting for (i) pressure dependent change in the alveolar volume, (ii) viscoelastic nature of the alveolar membrane, (iii) surface tension of the tissue-air interface and its dependence on surfactant secretion (alveolus volume dependent), and (iv) change in thickness of the alveolar membrane as the alveolus shrinks or expands. We focus in particular on the interplay between the air pressure signal time scale, oxygen diffusion time scale, tissue relaxation time scale, and blood pressure oscillation time scale.

Results
This research provides a robust model that examines the concentration of oxygen from the point the oscillatory air enters the alveolus to the point the pulmonary capillaries get oxygenated.

Conclusion
We expect this model to shed more light on the physics underlying the enhancement of the oxygenation process due to a wideband pressure signal.

Keywords: alveolar oxygenation, visco-elastic model of alveolus, pulmonary capillaries, perturbed air pressure, Fourier number for mass transfer

ABSTRACT #46

WITHIN THE ORBIT OF SCIENCE BUT NOT QUITE: NON-ACADEMIC BOOKS ABOUT SCIENCE

Dr. Samira Kaissi, Managing Director, Basic Science Research, Faculty of Medicine

Almost every research lab you enter has a Lehninger's Principles of Biochemistry or an Albert's Molecular Biology of the Cell on its shelves, but rarely do we see a book about the industry of research, its history or its effect on our everyday lives. Biographies about great scientists, books written by scientists who found out they were also good writers, or great writers who tackle some of science's most profound questions should all be part of a basic scientist's education. These non-didactic books about science and scientists open a window to the vast international community of scientists we all belong too, and allow us to see the far reaching effects of the everyday grinding benchwork that we are all too familiar with.

My poster will introduce 8 such books with a brief description of the book, why it was chosen, and its author. Some books are recent and others classic. This in the hopes that these great authors and topics will whet the appetite of some students and faculty to this genre, and to inspire. The books I chose so far are:

1. Nature via Nurture: Genes, Experience and what makes us Human (by Matt Ridley)
2. Rosalind Franklin: The Dark Lady of DNA (by Brenda Maddox)
3. Guns, Germs and Steel: The fates of Human Societies (By Jared Diamond)
4. The Where, the why and the how: 75 artists illustrate the wondrous mysteries of science
5. Ignorance: How it drives Science (by Stuart Firestein)
6. A book by Stephen Jay Gould who is of course is the fantastic writer and biologist who introduced the world to evolutionary biology in a way that no one else could. Book title to be decided.
7. The Great Influenza: the story of the deadliest pandemic in History (by John Barry)
8. Drug Discovery and Development: Technology in Transition (Raymond J Hill)

ABSTRACT #47

SIMVASTATIN REDUCES THE ZYMOSAN-INDUCED INFLAMMATORY RESPONSE IN A MOUSE AIR POUCH MODEL VIA HEME OXYGENASE-1 AND CYCLOOXYGENASE-2

Ghewa El Achkar[1,2], May F. Mrad[1], Eva Hamade[2] and Aïda Habib[1]

1. American University of Beirut, Department of Biochemistry and Molecular Genetics
2. Genomique et Sante, Doctoral School of Sciences and Technologies, Faculty of Sciences 1, Lebanese University, Lebanon
*Email: ghewaelachkar@hotmail.com

Background and aims
Statins have multiple pleitotropic anti-oxidant and anti-inflammatory effects. Studies showed *in vitro* an inhibition of the inflammatory cyclooxygenase-2 as well as an increase in the anti-oxidant heme oxygenase -1 in response to statins. We tested the effect of statins *in vivo* model of inflammation, the zymosan-air-pouch.

Methods
Dorsal air pouches were established in C57BL/6 mice by injection of 5 ml of sterile air, twice a week. Mice were treated with intra peritoneal injection of simvastatin daily for 2 days-20mg/Kg or 10 days-5mg/Kg prior to the injection of zymozan. 24 hours later, exudates were collected, leukocytes present in exudates were measured and prostaglandin E_2, interleukin-6, cyclooxygenase-2 and heme oxygenase-1 expressions were assessed.

Results
Short administration of simvastatin (2 days) inhibited cellular recruitment in response to zymosan by 51% ($p=0.016$, paired t-test), decreased interleukin-6 by 52% ($p= 0.036$, paired t-test), induced heme oxygenase-1 and inhibited cyclooxygenase-2 protein expression. Longer administration (10 days) of simvastatin showed a further decrease in cyclooxygenase-2 expression and in prostaglandin E_2 by 61% ($p<0.001$, paired t-test). The anti-inflammatory agent dexamethasone was used as a positive control, showing 27% inhibition of cellular recruitment ($p=0.002$, paired t-test), decrease in prostaglandin E_2 by 51% ($p=0.05$, paired t-test) and in interleukin-6 by 97% ($p=0.038$, paired t-test), induced heme oxygenase-1 and inhibited cyclooxygenase-2 protein expression.

Conclusion
Simvastatin exerts some of its anti-inflammatory effect through inhibition of cyclooxygenase-2 with a possible anti-oxidant effect through induction of heme oxygenase-1.
Future investigations will be performed to understand the role of these enzymes and the intra cellular mechanisms involved.

Keywords: inflammation, statins, heme oxygenase-1, cylooxygenase-2.

ABSTRACT # 48

Involvement of Renal Cytochrome P450 and Arachidonic Acid Metabolites in Diabetic Nephropathy

Stephanie Eid[1*], Ghina Noureddine[1], Kawthar Braych[1], Suzanne Boutary[1], Ayad Jaffa[2], Fuad Ziyadeh[3], Assaad Eid[1*]
[1]Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine at AUB,
[2]Department of Biochemistry and Molecular Genetics, Faculty of Medicine at AUB, [3]Department of Internal Medicine, Faculty of Medicine at AUB.
*PhD. student (Presenter) #Principal Investigator and Advisor

Background and aims: Diabetic nephropathy (DN), a serious complication of diabetes, is characterized by hyperfiltration, hypertrophy, extracellular matrix accumulation, fibrosis and proteinuria leading to loss of renal function. In renal hypertrophy, tubules increase in size and accumulate extracellular matrix and are also associated with alterations in renal sodium handling as well as hypertension; processes linked by involvement of the arachidonic acid (AA) metabolites 20-HETE and EETs. This study aims to determine the specific AA-metabolizing CYP450 isoforms present in proximal tubules (PT) that are altered by high glucose (HG) in cultured PTs, and in an animal model of diabetes. It intends to investigate the effects of alterations in CYP isoforms and/or AA-metabolite levels in DN. This work will investigate the mechanism of PTs injury and the effect of inhibition of AA-metabolites *in vitro* and will also get insight onto the cross-talk between CYP450 isoforms and other sources of Reactive Oxygen Species (ROS).

Methods: Immunohistochemistry, hypertrophy, apoptosis, fibrosis, ROS generation, 20-HETE and EET formation, CYP4A and Nox protein expression, and mRNA levels were measured in vitro and in vivo.

Results: Exposure of PT cells to HG resulted in apoptosis and hypertrophy. HG treatment increased ROS production and was associated with CYP4A and CYP2C upregulation, 20-HETE and EETs formation, and Nox oxidases upregulation. The effects of HG on Nox proteins and mRNA expression, matrix protein accumulation and apoptosis were blocked by HET0016, an inhibitor of CYP4A, and were mimicked by 20-HETE. Inhibition of EETs *in vitro* promoted the effects of HG on cultured proximal tubular cells. In parallel, the levels of CYPs 2B, 2C, and 4A were assessed in a rat model of streptozotocin-induced diabetes. There was significant induction of expression and activity over control of these CYPs in *vivo* associated with an increase in ROS production, Noxs expression, PTs injury, and this was prevented by insulin therapy.

Conclusion: Our results indicate that hyperglycemia in diabetes has a significant effect on the expression of AA-metabolizing CYPs, manifested by increased AA metabolism, and might thus alter kidney function through alteration of type and amount of AA metabolites; this pathway is through an oxidative stress-dependant mechanism.

Key words: Diabetic Nephropathy, Cytochromes P450, Arachidonic Acid metabolites, NADPH oxidases, Reactive oxygen species.

ABSTRACT # 49

Novel Mechanistic Pathways Linking Diabetes to Colorectal Cancer

Joanna Fares[1*], Sarah Abou-Merhi[1], Kawthar Braych[1], Suzanne Boutary[1], Fuad Ziyadeh[2], Ayad Jaffa[3], Assaad Eid[1#]
From the [1]Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine at AUB, and the [2]Department of Internal Medicine, Faculty of Medicine at AUB, and the Department of Biochemistry and Molecular Genetics, Faculty of Medicine at AUB.
*Postdoctoral Fellow (Presenter)
#Principal Investigator and Advisor

Background and Objectives: Both diabetes and cancer are prevalent diseases whose incidence is increasing worldwide and especially in countries that are undergoing rapid industrialization (i.e. Golf Countries). Epidemiological studies provide strong evidence that subjects with diabetes are at significantly higher risk of developing many forms of cancer and especially solid tumors including colorectal cancer. While diabetes and cancer share many risk factors, the biological links between the two diseases are poorly characterized. In this study we will determine the role of AMPK and mTOR and their crosstalk with the NADPH oxidases in normal and cancerous colon epithelial cells and their response to high glucose (HG), high insulin (HI) and their combination. We will also explore the mechanism by which diabetes accelerates tumor development and tumor burden.

Methods: Cancer migration, cancer proliferation, cancer invasion, immunohystochemistry, Noxs, fibronectin, AMPK, mTOR proteins expression and ROS production were performed in this study.

Results: We have evidence that HG or HI induces reactive oxygen species (ROS) production in both normal human epithelial colon cells (NCM356) and human epithelial colon adenocarcinoma cells (CaCo2). To a greater interest, colon cancer itself is a major source of ROS production. Treating CaCo2 cancerous cells with either HG or HI inactivates adenine monophosphate kinase (AMPK), up-regulates NADPH oxidases Nox1 and Nox4-induced ROS production, associated with increased fibronectin expression, induces the loss of function of the tumor suppressor gene, tuberous sclerosis complex 2, encoding tuberin, and activates the mTOR pathway. In addition, HG or HI enhances cancer cell migration, proliferation, and invasion. Pharmacologic activation of AMPK by 5-aminoimidazole-4-carboxamide-1-riboside AICAR or inhibition of mTOR by rapamycin restores AMPK and tuberin phosphorylation/activity, down-regulates Nox1, Nox4, and fibronectin expression, increases mTOR phosphorylation/activity, and regulates cell migration, proliferation, and invasion

Conclusion: Our results uncover a novel and critical role for AMPK and mTOR in cancer cell proliferation and extracellular matrix accumulation in the diabetic milieu; this pathway is through an oxidative stress-dependant mechanism. Our work will set the stage for additional studies to explore new therapeutic approaches for the treatment of cancer in diabetic patient and maybe to a larger extent treatment of cancer.

Key words: Diabetes, colorectal cancer, Reactive oxygen species, AMPK/mTOR pathway.

ABSTRACT # 50

The Role and Sources of ROS in Cigarette Smoke-Induced Kidney Injury

Firas Kreidieh[1*], Suzanne Boutary[1], Ahmad Husari[2], Assaad Eid[1#]

From the [1]Department of Anatomy, Cell Biology and Physiology, Faculty of Medicine at AUB, and the [2]Department of Internal Medicine, Faculty of Medicine at AUB.
*Med IV student (Presenter)
#Principal Investigator and Advisor

Introduction: Cigarette smoking has been identified as the most important source of preventable morbidity and mortality in the United States (A, B). Not only it is a major risk factor for atherosclerotic vascular diseases including stroke and coronary artery disease (C), but also it increases the risk for chronic obstructive pulmonary disease (C) and for various forms of cancer (D, E) and is a risk factor in the progression of chronic kidney disease (CKD) (F). There is evidence that nicotinamide adenine dinucleotide phosphate (NADPH) oxidases of the Nox family contribute to the oxidative stress associated with smoking (L). The scarcity of studies on one hand, combined with the importance of smoking as a preventable source of morbidity and mortality on the other, encouraged us to study further the role of oxidative stress in smoke-induced nephropathy, more importantly to identify the source of ROS playing the major role in cigarette smoke-induced kidney injury. To do so, we studied the expression of the two different isoforms, Nox1 and Nox4, by podocytes treated with smoke extract.

Materials: Smoke extraction, podocytes culture and treatment, Western Blot Analysis

Results: Exposure of mouse podocytes to different concentrations of smoke extract resulted in different effects on NADPH oxidase expression. Nox 1 expression was upregulated when podocytes were exposed to a CSES concentration equal to or greater than 3%. This is consistent with the observation that the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases of the Nox family contribute to the oxidative stress associated with smoking (L). On the other hand, expression of Nox 4 was the same under all treatment conditions, including the control. Although this result is not consistent with the previously mentioned observation, it is not unusual and agrees with the fact that current data on the expression of the Nox family of proteins in cells are somewhat contradictory (O).

Conclusion: Cigarette Smoke Extract is involved in kidney injury, especially podocyte injury, by inducing production of reactive oxygen species. According to what our results have shown so far, this role seems to be more through upregulation of Nox 1 expression and not Nox 4. It would be interesting to study its crosstalk with the AMPK/mTOR pathway as well.

Keywords: kidney injury**,** oxidative stress, NADPH oxidases, Cigarette smoke extracts.