Scientific Tracks & Abstracts
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BIOCHEMISTRY, MOLECULAR BIOLOGY: R&D

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A non-conventional therapeutic approach to epilepsy in children

Mariana Babayeva
Touro College of Pharmacy, USA

Children epilepsy is a complex disease with a variety of distinct syndromes. Treatment of pediatric epilepsy is challenging. Childhood epilepsies are commonly associated with seizures that are resistant to existing treatment methods. Therefore, treatment of pediatric epilepsy requires more effective therapy to avoid short-term and long-term neurological disorders. Marijuana has been used to treat disease since ancient times. Marijuana ingredients Cannabidiol (CBD) and D9-Tetrahydrocannabinol (THC) have created a significant research interest as potential therapy options in epilepsy treatment. THC is the major psychoactive component of marijuana that aids in reducing epileptic seizures. CBD has proven to have anticonvulsant effect not only in experimental models but also in clinical studies. Research studies have provided strong evidence for safety and anticonvulsant properties of medical marijuana. Principal concerns regarding the use of medical marijuana in children include lack of standardization and regulation, imprecise dosing, possible adverse side effects and medication interactions.

Speaker Biography

Mariana Babayeva is an Associate Professor at Touro College of Pharmacy, New York. In addition to her role at Touro, she is also an Adjunct Professor at Rockefeller University and Visiting Scientist at Arnold and Marie Schwartz School of Pharmacy of LIU. She has over 14 years of experience in clinical practice. She is recognized for her expertise in the pharmacokinetics and the use of animal and organ models. She has conducted several international research projects. She has been published in peer-reviewed journals and serving as an Editorial Board Member of repute.

e: mariana_babayeva@yahoo.com

Notes:

Small molecule pro-neurotrophic therapeutic activity in murine models of Alzheimer's disease

Stuart Maudsley
University of Antwerp, Belgium

Age-related neurodegenerative diseases, such as Alzheimer's disease, will represent one of the largest future burdens on worldwide healthcare systems due to the increasing proportion of elderly in our society. As deficiencies in neurotrophins are implicated in the pathogenesis of many age-related neurodegenerative disorders, it is reasonable to consider that global neurotrophin resistance may also become a major healthcare threat. Central nervous system networks are effectively maintained through aging by neuroprotective and neuroplasticity signaling mechanisms which are predominantly controlled by neurotrophin receptor signaling. Neurotrophin receptors are single pass receptor tyrosine kinases that form dimeric structures upon ligand binding to initiate cellular signaling events that control many protective and plasticity-related pathways. Declining functionality of the neurotrophin ligand–receptor system is considered one of the hallmarks of neuropathological aging. Therefore, it is imperative to develop effective therapeutic strategies to contend with this significant issue. The development of nonpeptidergic, small-molecule ligands can overcome these limitations, and productively regulate this important receptor system with beneficial effects. We have found that in multiple models of Alzheimer's disease the previously employed anti-depressant Elavil can exert potent pro-neurotrophic activity through a series of complementary mechanisms. This small molecular agent possesses the capacity to significantly enhance cognitive performance in mouse models possessing considerable levels of dementia and amyloid pathology. In this respect agents such as Elavil may represent an important addition to a new wave of therapeutic strategies against dementia.

e: stuart.maudsley@uantwerpen.vib.be

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Strategies for pathology-activated generation of reelin trafficking modulators for altering late-onset Alzheimer's disease progression

Ronald A Hill and **Amal K Kaddoumi**
University of Louisiana, USA

Compelling evidence continues to accrue that late-onset (non-familial) Alzheimer's disease (LOAD), arising early in olfactory structures of the brain, progresses in a spatio-temporally consistent pattern via propogating "inflammaging" processes; predisposing susceptibility may be established as early as gestationally. Genome-wide association studies (GWASs) of cognitively uncompromised individuals who exhibit apparent high pathology suggest that select combinations of genetic attributes can confer resistance to cognitive decline; and, although certain specifics of pathological signatures differ from those in cognitively deteriorating individuals, these might reflect successful defense against an otherwise pathological chain of events. A central player emerging from these GWASs is reelin, a large glycoprotein component of the extracellular matrix (ECM). Though historically regarded mostly as a key player in embryo-fetal development reelin exhibits functional interplay in the adult brain with other molecular constituents having clearly established associations with LOAD, such as apolipoprotein E, and with the responsiveness states of inflammaging-associated cells, notably microglia. The complexities and relative paucity of knowledge regarding ECM maintenance, remodeling, and functional dynamics, and the analytical challenges involved in achieving increased clarity, means that gaining therapeutically actionable traction will be difficult; however, the tantalizing thought that GWASs of these individuals may be showing us ways forward motivates rising to these challenges. Reelin possesses multiple functional domains, and certain reelin fragments exhibit trafficking and function disparate from intact glycoprotein. Very recently, ADAMTS-3 was identified as the catalyst of a proteolytic cleavage of reelin shown to be inactivating in terms of reelin's canonical activities. Because of reelin remains heavily involved during adulthood in dynamic brain maintenance and remodeling, deleterious consequences can be expected from anatomically untargeted alterations in reelin function. Potential strategies for pathology-activated, localized generation of suitable modulators will thus be needed, and progress with respect to devising such strategies will be shared in this presentation.

Speaker Biography

Ronald A Hill is now spanning almost 35 years, over a pharmaceutical sciences career. He has aimed to become a generalist with high-level acumen in relating the Chemistry of biologically active molecules to their interactions with, and actions on, humans and their hosted organisms (normal microbiome, microbial pathogens, parasites). The central focus of his own research has always related to the CNS, guided also by the aim of constantly gaining acumen in molecular therapeutics design, molecular biopharmaceutics, and molecular toxicology and in general, the science of successfully bridging discovery at its earliest stages to clinical application. His educating duties at the PhD and PharmD levels are extensive, and carried out with the underlying hope that the molecular science and molecular design will be intelligently and artfully acted on in clinical practice. His current research collaborations center on neurodegenerative conditions and cancer.

e: rhill@ulm.edu

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Effect of manufacturing process and container closure configurations on critical quality attributes of generic parenteral drug product: A case study of pH

Lakshmi Prasanna Kolluru
Medefil Inc., USA

Purpose/Introduction: Product development of generic parenteral products involves extensive studies to optimize formulation process and manufacturing conditions such that the developed product is like Reference Listed Drug (RLD) in terms of all Critical Quality Attributes (CQA) over stability. CQA's are properties of drug product which have significant effect on product quality. Typical CQA's for a drug product include pH of the formulation, assay of the chemical of interest, impurities, visual appearance, particulate matter, color and clarity of the solution. In this present work, we present two case studies of generic parenteral product development to optimize pH of the drug products under study.

Methods: pH of a formulation is a critical quality attribute as it significantly affects solubility and stability of the drug product. In addition, pH of a parenteral drug product has significant clinical effects such as electrolyte imbalances. In first case study, we evaluated effect of container closure configurations on pH of the drug product. We formulated the drug product and filled it in various treated and non-treated glass vials, stoppered, sealed and placed on stability as per International Conference on Harmonization (ICH) guidelines. In the second case study, we evaluated effect of nitrogen sparging during compounding on pH of the drug product. We prepared three batches of drug product. First batch was prepared under ambient atmospheric conditions without nitrogen sparging, another by sparging the water for 30 minutes before addition of Active Pharmaceutical Ingredient (API) and blanketing the formulation with nitrogen for rest of compounding process; and third batch with continuous nitrogen sparging throughout compounding. pH of all the three batches are monitored at pre-determined time intervals throughout the manufacturing process.

Results: Stability data of the drug product in case study 1 monitored over 6 months at 25C/65% RH and 40C/75% RH suggest that the formulation in untreated vials showed drastic change in pH, with the data at 3M and 6M even failing to meet pH specifications for the finished product. However, formulation filled in treated vials has well controlled pH and within specifications at all conditions up to 6M on stability. Data analysis of various batches from case study 2 suggests better control of pH in the second batch with sparging the water for 30 minutes before addition of nitrogen and blanketing throughout the compounding process.

Conclusion: Both the case studies suggest that appropriate container closures and optimal manufacturing process have significant effect on pH of parenteral drug products and should be closely evaluated during product development.

Speaker Biography

Lakshmi Prasanna Kolluru is currently working as Sr. Formulation Scientist at Medefil, Inc, a generic pharmaceutical company. She is responsible for leading product development project teams all the way from kick-off to product approval. Prior to joining Medefil, she has served in formulation, analytical and clinical development groups across brand pharma, generic pharma and contract research organizations. She has graduated with a PhD in Pharmaceutical Sciences from Mercer University, Atlanta, GA. Her thesis research focusing on development of novel targeted drug delivery system for tumor theragnosis has been recognized internationally by American Association of Pharmaceutical Scientists (AAPS) for excellence in graduate research. In addition to her active research, she serves as Editorial Board Member and Peer-Reviewer for several international journals.

e: prasanna.kolluru@medefilinc.com

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Investigation of the biochemical mechanism of action of antioxidants in the prevention of cancer

Kissi Mudie
Ethiopian Public Health Institute, Ethiopia

Background: The safe use of medicines is a critical issue for all health care professionals. Cancer refers to a group of diseases that are associated with a disturbance in the control of cell growth and metabolism. Indeed, the unbalanced control of cellular proliferation is a primary characteristic of cancer cells and, as such, any molecule capable of inhibiting cancer cell proliferation may also be useful as a potential chemo-preventive agent. Throughout history, antioxidants have been the most significant source of anticancer and chemopreventing agents. More than 1,000 different phytochemicals are already proved to possess interesting chemopreventing activities. Antioxidants consist of a wide variety of biologically active phytochemicals including phenolics, flavonoids, carotenoids, etc. that have been shown to suppress early and late stages of carcinogenesis.

Objective: The objective of this study was to review recent biochemical and molecular mechanisms, in relation to natural and synthetic chemopreventing substances (antioxidants) for cancer control and management.

Findings: Antioxidants exert anticancer effects via a variety of mechanisms, including removal of carcinogenic agents, modulation of cancer cell signaling and cell cycle progression, promotion of apoptosis and modulation of enzymatic activities.

Conclusion: This review provides an updated and comprehensive overview on the anticancer effects of antioxidants *in-vitro* and *in-vivo* animal models including recent intervention studies. Finally, possible mechanisms of action involving antioxidant and pro-oxidant activity as well as interference with cellular functions are discussed.

Speaker Biography

Kissi Mudie has completed his MSc in Medical Biochemistry from Addis Ababa University, School of Medicine. He is the Director of National clinical chemistry laboratory, Ethiopian Public Health Institute. He has published more than 16 papers in reputed journals and has been serving as Researcher.

e: kissimudiey@yahoo.com

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Nanostructured Lipid Carriers (NLC)-Based gel for the topical delivery of azelaic acid: Designing, characterization and *in-vitro* evaluation

Deepinder Singh Malik and **Gurpreet Kaur**
Punjab University, India

Azelaic acid (AZA) is a naturally occurring dicarboxylic acid, reported to be effective in management of mild to moderate acne vulgaris. However, few noticeable dose-related side effects limit its therapeutic applicability. Therefore, the study was directed towards the optimization, formulation and evaluation of the AZA loaded nano-structured lipid carrier (NLCs) to enhance its payloads and achieve sustained release at the target site. NLCs were prepared by melt emulsification and ultra-sonication method employing glyceryl monostearate and oleic acid as solid and liquid lipid, respectively. The formulation was optimized employing design expert software taking sonication time, amplitude and drug concentration as independent variables with particle size and drug entrapment as dependent variables. The optimized preparation so formed was incorporated into aloe-vera based carbopol gel and evaluated for its size, morphology, pharmacokinetic and pharmacodynamic parameters. NLCs were found to possess mean particle size in a range of 45-48 nm with low polydispersity index value (~ 0.4) and encapsulation efficiency of ca. 82%. It was further verified employing transmission electron microscopy which depicted the formation of uniform surfaced spherical nanoparticles. *In-vitro* permeation and skin retention studies revealed significant retention of AZA within the skin with minimum penetration across the skin. Draize patch test exhibited no signs of irritation/lesion on the skin indicating its non-irritating nature. Skin distribution analysis employing rhodamine 6G as a fluorescent dye unveiled the deposition of NLCs preparation to the deeper layers of skin. Thus, as per experimental findings, NLCs may be explored as promising carriers for site specific targeting.

e: deepinder.malik88@gmail.com

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Regulation of the activity of the promoter of RNA-induced Silencing, C3PO

Suzanne Scarlata
Worcester Polytechnic Institute, USA

RNA-induced silencing is a process which allows cells to regulate the synthesis of specific proteins. RNA silencing is promoted by the protein C3PO (component 3 of RISC). We have previously found that phospholipase Cβ, which increases intracellular calcium levels in response to specific G protein signals, inhibits C3PO activity towards certain genes. Understanding the parameters that control C3PO activity and which genes are impacted by G protein activation would help predict, which genes are more vulnerable to down-regulation? Here, using a library of 1018 oligonucleotides, we show that C3PO binds oligonucleotides with structural specificity but little sequence specificity. Alternately, the rate of hydrolysis is exquisitely sensitive to the substrate stability. Importantly, we find that oligonucleotides with higher Tm values are inhibited by bound PLCβ. This finding is supported by microarray analysis in cells over-expressing PLCβ1. Taken together our work enables predictions of the genes whose post-transcriptional regulation is responsive to the G protein/phospholipase Cβ/calcium signaling pathway.

Speaker Biography

Suzanne Scarlata is a Professor Emeritus of Stony Brook University and a Whitcomb Chair at Worcester Polytechnic Institute. Most of her research has focused on the regulation of G protein signaling in model systems and in cultured cells using primarily fluorescence methods. The work presented here represents an unexpected connection between the impact of extrasensory information and post-transcriptional gene regulation through the Gαq/phospholipase Cβ signaling pathway.

e: sfscarlata@wpi.edu

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Physical forces cause *HoxD* gene cluster elongation

Spyros Papageorgiou
National Center for Scientific Research-Demokritos, Greece

Hox gene collinearity is a fundamental property in the process of *Hox* gene expression. It correlates the 3' to 5' sequential gene alignment in the *Hox* gene cluster with the ontogenetic units along the anterior/posterior axis of the embryo. This property is multiscalar and cannot be treated by biomolecular mechanisms alone. In multiscale phenomena physical laws must come into play. The biophysical model (BM) provides the necessary tools for an integrated multiscalar explanation of *Hox* collinearity. According to BM, physical forces are created which pull the *Hox* genes sequentially from the compact inactive *Hox* gene cluster toward the transcription factory domain, where gene transcription is possible. The BM successfully describes the genetic engineering experiments where some genes of the vertebrate *Hox* cluster are deleted (or duplicated). Although the BM was introduced in 2001, it is only in the last 2 years that it has been adopted by the scientific community, because the evidence was missing for the existence of such forces. However, recent instrumental progress in achieving high imaging resolution (e.g. 3D DNA FISH, STORM etc.) make possible the confirmation of several BM predictions. For instance, it is found that the mouse *HoxD* cluster is elongated up to 5-6 times during *Hox* gene transcription. These unexpected physical deformations agree with the BM predictions. New experiments are proposed to test further the biophysical model. A synthesis of Biophysics and Biochemistry is proposed to explain *Hox* gene collinearity in two steps: in a first step, the BM forces translocate the *Hox* genes in the right location for transcription. In a second step, biomolecular mechanisms transcribe the translocated genes.

Speaker Biography

Spyros Papageorgiou has graduated in Physics from the Athens University, Greece. He has received his DPhil in Theoretical Physics from Oxford and Sussex Universities in 1965. He was a Research Fellow at Theory Division of CERN 1968-1970 and a Corresponding Fellow between CERN and Demokritos, Greece 1970-1973. In 1976, he started working on models in Developmental Biology. He formulated models in reaction-diffusion, pattern regulation, regeneration, gene expression etc. In 2000, he became Emeritus Research Director at 'Demokritos' and he currently study the *Hox* gene collinearity problem. He formulated the biophysical model (BM) (S Papageorgiou, BIOLOGY 2017,6, 32) based on the hypothesis of physical forces translocating the *Hox* genes toward the transcription factory domain where transcription is possible.

e: spapage@bio.demokritos.gr

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Neuron-specific regulation of alternative pre-mRNA splicing

Sika Zheng
University of California, Riverside, USA

Families of alternative splicing regulators often contain multiple paralogs presumed to fulfill different functions. Polypyrimidine tract binding proteins *Ptbp1* and *Ptbp2* exhibit dynamic stage-specific expression and program developmental pre-mRNA splicing in neurons, but how and why their regulatory actions differ are not understood. To compare their targeting, we generated a knockin mouse allele that conditionally expresses *Ptbp1*. Bred to a *Ptbp2* knockout, the transgene allowed us to compare the developmental and molecular phenotypes of mice expressing only *Ptbp1*, only *Ptbp2*, or neither protein in the brain. This knockin *Ptbp1* rescued a forebrain-specific, but not a pan-neuronal, *Ptbp2* knockout, demonstrating both redundant and distinct roles for the proteins. Using comprehensive approaches of biochemistry, RNA-Seq, and CLIP-Seq to probe their targeting and protein-RNA interactions, we found that many developmentally regulated exons exhibited different sensitivities to *Ptbp1* and *Ptbp2*. Nevertheless, the two paralogs displayed similar RNA binding across the transcriptome, indicating that their differential targeting does not derive from their RNA interactions, but from possible different cofactor interactions.

Speaker Biography

Sika Zheng is an expert in studying RNA binding proteins and alternative splicing. His lab combines Biochemistry, Molecular Biology, Cell Biology, Neurobiology, Genetics, Genomics, and Computational Biology to understand the activity, mechanism, function and dysfunction of gene regulation at the RNA level in the nervous system focusing on *Ptbp1* and *Ptbp2* two RNA binding proteins programming neuron-specific alternative splicing. He has made seminal contributions revealing the roles of alternative splicing for neuronal development and the mechanisms of *Ptbp1*/2 controlling neuron-specific alternative splicing events.

e: sika.zheng@ucr.edu

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The new role of lipin1 in myogenic progenitor differentiation to muscle and adipose tissues

Hongmei Ren
Wright State University, USA

Brown adipose tissue and skeletal muscle originate from common myogenic factor 5-expressing (Myf5) progenitors. Despite, the great promise of directing Myf5+ progenitor cells in the treatment of obesity, little is known about what controls the progenitors commit to the brown adipogenic lineage. Lipin1 catalyzes the penultimate step in triglyceride synthesis and play an important role in promoting adipogenic differentiation in Myf5neg fibroblast cells. Surprisingly, depletion of lipin1 in Myf5+ progenitors in our newly generated Lipin1Myf5cKO mice promotes brown adipose tissue conversion indicated by inhibition of skeletal muscle development and expanded brown adipose tissue formation in the dorsal cervical region compared to control littermates. In this seminar, I will discuss about the mechanism of lipin1 in regulating skeletal muscle development and commitment and differentiation of skeletal muscle and brown adipose tissue, and affects the cell fate switch between myogenesis and adipogenesis.

Speaker Biography

Hongmei Ren is currently an Assistant Professor in the Department of Biochemistry and Molecular Biology at the Wright State University. She has received her Postdoctoral training in Cardiovascular Research Center at University of Kentucky. Her research interests focus on lipid metabolism, and their effects on cardiac and skeletal muscle function. Her laboratory recently revealed a previously unknown role of lipin1 (phosphatidic acid phosphatase) in controlling myogenic cell fate commitment.

e: Hongmei.ren@wright.edu

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Hydralazine induces stress resistance and extends lifespan in *C. elegans* via Nrf2/SKN-1 pathway

Hamid Mirzaei
University of Texas Southwestern Medical Center, USA

Oxidative stress increases gradually with aging and steadily diminishes the cell's ability to maintain homeostasis. Nuclear factor (erythroid-derived 2)-like 2 and its *C. elegans* ortholog, SKN-1, are transcription factors that play a pivotal role in the oxidative stress response, cellular homeostasis and lifespan. But like other defense systems, the Nrf2-mediated stress response is compromised in aging and neurodegenerative diseases. In this study, we provide evidence that hydralazine, a drug used for treatment of hypertension, is a bona fide activator of the Nrf2/SKN-1 pathway. We demonstrate that hydralazine protects Alzheimer's disease model cells and *C. elegans* from chemical stressors linked to neurodegenerative diseases. We also show that hydralazine extends lifespan and health in *C. elegans*. Hydralazine is an FDA approved drug; therefore, we suggest it is an excellent candidate for clinical trials for treatment of age-related disorders. Hydralazine may also offer general health benefits for the aging population.

Speaker Biography

Hamid Mirzaei's research is focused on finding the target of novel and FDA approved compounds using a combination of Proteomics, Computational Biology and Biochemistry. Many FDA approved drugs are currently in use without clear understanding of their mechanism of action. On the other hand there are quite a few well-characterized natural products with unknown targets. His research is focused on understanding the drug's mechanism of action by identifying the target of the drugs and their cellular and organismal phenotypes.

e: hamid.mirzaei@utsouthwestern.edu

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Nanomaterial regulates the radiosensitivity in colorectal cancer cells

Shengfang Ge
Shanghai JiaoTong University School of Medicine, China

Introduction: Colorectal cancer (CRC) is a common gastrointestinal malignant tumor with high rate of postoperative recurrence. And the risk of metastasis of CRC is still one of the main reasons for the failure of CRC treatments. Radiation therapy is a commonly method to treat CRC, which occupies an irreplaceable important position in surgery, chemotherapy and other treatments. Metal-based nanomaterial was deemed as one of the radio sensitivity agent due to atom effect.

Objective: To improve the effects of irradiation on tumor cells, we testified the effect of Graphene Quantum Dots (GQDs) with good biocompatibility and rich oxygen groups on radio sensitivity. Meanwhile, we investigated the radio sensitivity mechanism of GQDs. Our study would provide reliable experimental basis for GQDs as a radiotherapy sensitization agent in potential clinical applications.

Contents & Methods: The GQDs were prepared with graphene oxide (GO) and the safe concentration of GQDs were determined by CCK8 assay, laser confocal microscope and transmission electron microscopy are carried out to measure the sub-cellular localization of GQDs, the proliferation ability of different treated groups were detected by performing CCK8 assay and colony formation assay, the cell apoptosis rate and the cell cycle arrest of treated groups were detected by Flow Cytometry, the cell damage was observed by transmission electron microscopy, the production of ROS and mitochondrial ROS in treated groups were measured by DCFH-DA and MITOSOX Red Indicator, respectively, the expression of γH2AX which reflect the degree of DNA double-strand breaks was detected by western blot after different treatments.

Results: The safety concentration of GQDs on SW620 and HCT116 cells was 50 μg/mL. Transmission electron microscopy and laser confocal microscope revealed that GQDs were mainly distributed in cytoplasm of cells. In addition, our study indicated that GQDs could decrease the cell viability, increase the degree of cell damage and cell apoptosis of SW620 and HCT116 cells under the irradiation synergistic effects. Meanwhile, with the synergistic effects of ionizing radiation, GQDs could enhance intracellular ROS generation of SW620 and HCT116, and increased the ROS levels in mitochondria which increase DNA double-strand break out and G2/M phase cell cycle arrest cells.

Conclusions: This study demonstrated that GQDs have good radio sensitivity at cell levels *in vitro*, which can improve the killing effects of irradiation on tumor cells, and ultimately achieving treating cancer. It illustrates that GQDs present great potentials in tumor therapy as a new type of radio sensitivity agent. In this seminar, I will discuss the protocol we developed to pattern the first human hNT neurons on parylene-C/ SiO2 substrates and how, in our more recent work, we have patterned the first hNT astrocyte, on such substrates to single cell resolution.

e: geshengfang@sjtu.edu.cn

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Molecular changes in penumbra after focal photothrombotic stroke in the rat cerebral cortex

Anatoly B Uzdensky and **Demyanenko S V**
Southern Federal University, Russia

In ischemic stroke cell damage propagates from infarct core to surrounding tissue. To reveal proteins involved in neurodegeneration and neuroprotection in penumbra, we studied biochemical consequences of focal photothrombotic infarct (PTI) in the rat cerebral cortex. Photosensitizer Bengal Rose does not cross blood brain barrier and remains in vasculature. Following laser irradiation induces focal vessel occlusion and brain cortex infarct. Using proteomic microarrays "Panorama Ab Microarray, Cell Signaling" and "Panorama Ab Microarray, Neurobiology" (Sigma-Aldrich), we studied expression of 448 proteins in penumbra at 1, 4 or 24 hours after PTI as compared with untreated contralateral cortex. Diverse cellular subsystems were involved in penumbra response to PTI: (1) Proteins initiating, regulating or executing various apoptosis stages (caspases 3, 6, 7, SMAC/DIABLO, Bcl-10, Par4, E2F1, p75, p38, JNK, p53, NMDAR2a, c-myc); (2) Anti-apoptotic proteins (Bcl-x, p63, MDM2, p21WAF-1, ERK1/2, ERK5, PKCα, PKCβ, PKCμ, RAF1, phosphatases 1α and MKP-1, calmodulin, CaMKIIα, CaMKIV, estrogen and EGF receptors), (3) Signaling proteins (protein kinases Bα, GSK-3, PKC, DYRK1A, TDP43, phospholipase Cγ1, S-100, axin1, GSK-3, FRAT1, NUMB); (4) Proliferation regulators (Cdk6, Cdc7 kinase, Trf1, topoisomerase-1); (5) Axon outgrowth and guidance (NAV-3, CRMP2, PKCβ2); (6) Intercellular interactions (N-cadherin, PMP22); (7) Regulation of actin (cofilin, actopaxin, p120CTN, α-catenin, p35, neurofilament 68, neurofilament-M, ezrin, tropomyosin, spectrin (α+β), myosin Va and pFAK) and microtubule cytoskeleton (βIV-tubulin, polyglutamated β-tubulin, doublecortin, Tau, MAP1); (8) Vesicular transport and synaptic transmission (syntaxin-8, TMP21, Munc-18-3, synip, ALS2, VILIP1, syntaxin, synaptophysin, synaptotagmin, syntaxin, AP2β/γ, adaptin β1/2); (9) Biosynthesis of neuromediators (tryptophan hydroxylase, monoamine oxidase B, glutamate decarboxylase, tyrosine hydroxylase, DOPA decarboxylase, dopamine transporter); (10) ubiquitin-mediated proteolysis (ubiquilin-1, UCHL1, NEDD8); (11) Mitochondria quality control (Pink1, parkin, HtrA2); (12) Cytoprotection (AOP-1, MAKAPK2, chaperons Hsp70, Hsp90); (13) APP-related proteins (APP, β-amyloid, nicastrin). These data provide the integral view on cellular response in penumbra to PTI. They are involved either in neurodegeneration, or neuroprotection. These changes were highest at 4 h after PTI and reduced at the next day. Some of these proteins may serve as potential targets for ischemic stroke therapy.

Speaker Biography

Anatoly B. Uzdensky is a Professor in Biophysics and the Head of the Laboratory of Molecular Neurobiology at the Southern Federal University (Rostov-on-Don, Russia). He is the author of more than 120 journal papers and three books. His current research interests include stroke and neurotrauma, neurodegeneration and neuroprotection, cell biology, and proteomics.

e: auzd@yandex.ru

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The study on the effect of non-enzymatic glycation on the interaction of human serum albumin and sodium-fluorescein, via spectroscopic analyses

Priyankar Sen, Sadaf Fatima, Tamanna Anwar, Nabeel Ahmad and **Asimul Islam**
VIT University, India

The binding of SF to Human Serum Albumin (HSA) has been predicted by molecular docking and investigated by circular dichroism (CD) and fluorescence spectroscopy with or without glycation at temperatures 296K, 301K, and 310K. The binding parameters were calculated by quenching of emission spectrum of a constant concentration of SF (2 µM) at 513 nm against increasing concentrations of glycated or unmodified HSA as quencher starting from stochiometry ratio of 1:1. Sodium Fluorescein (SF) is a fluorescent tracer dye used extensively in diagnostic tools in the field of Ophthalmology, particularly in intravenous fluorescein angiography (IVFA). The HSA-SF interaction found to be a static binding. The Stern-Volmer constants (Ksv) were in the range of ~104 M-1 and other thermodynamic parameters like enthalpy (ΔH0), free energy (ΔG0) and entropy (ΔS0) are like albumin ligand bindings reported by previous workers. The interactions were found to be spontaneous, irrespective of temperature or glycation. Glycated HSA is clinically used to monitor unstable glycemic controls in diabetic patients. 39% increase in binding affinity (log K) and free energy (ΔG0) is reported on glycation at 310 K (room temperature), which may be important in the SF based angiographies. Further, on glycation HSA-SF binding seems to change from an enthalpy-driven to an entropy-driven reaction. SF shows best binding to FA binding site III of HSA, which also overlaps with drug binding site II of sub domain IIIA. Leu-430 seems to play a pivotal role in the interaction. This is the first report of glycated HSA and SF binding and comparison between the thermodynamic parameters of the bindings in the absence and presence of glycation at different temperatures.

Speaker Biography

Priyankar Sen is working in the field of protein folding and protein ligand interactions for the enhanced understanding of the molecular behavior of proteins, specifically albumins. He has done his PhD from Rizwan Khan's Lab in IBU, Aligarh Muslim University, India and Post-doctorate from Salunke's Lab, NII, New Delhi. Out of 5 years' Doctoral and 8 years Post-doctoral research, he has published 21 papers in international peer reviewed journals. He is currently working as Assistant Professor in VIT University. Currently, he is focusing on expression and modification of therapeutically important proteins and further scale up in bioreactors.

e: priyankar.sen@vit.ac.in

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In silico and *in vivo* assays of a borinic DOPA-derivative for Parkinson disease

Marvin A Soriano-Ursúa, Ana L Ocampo-Néstor, Antonio Abad-García and **José G Trujillo-Ferrara**
Instituto Politécnico Nacional, Mexico

The boron atom has some chemical properties which confer it advantages to be added in potential new drugs. One of these advantages has been inferred by *in silico* assays interaction on receptors or proteins with serine, threonine or tyrosine on the active site. In this sense, catecholamine receptors (belonging to the G-protein coupled receptors family) have a conserved binding site with three serine-residues involved in receptor activation. In this work, we tested the potential activity of 3-D models representing adducts of levodopa or dopamine on models of catecholamine human receptors (emphasis on beta-adrenoceptors and D2 and D3 receptors) by *in silico* docking analyses. Then, we synthesized and characterized a compound with potential activity on D2 receptor judged with the affinity score and binding mode on this receptor. Interestingly, the boron-containing compound contacts on the orthosteric site with higher affinity than Levodopa or dopamine, but its boron atom is not directed to serine residues in fifth transmembrane domain. This compound is an adduct of levodopa and an aryl-diphenylborinic acid, which was tested in a C57/BL6-mice model of parkinsonism induced by peritoneal administration of MPTP (a well-known toxin on catecholaminergic system). The compound induced improved performance of administered mice on motor tests but several pharmacological tests are required to elucidate the putative mechanism of action.

Speaker Biography

Marvin A Soriano-Ursúa has completed his PhD from Escuela Superior de Medicina del Instituto Politénico Nacional, México. He is a Member of the National System of Researchers, and he is Head of the Physiology Laboratory. He has focused on the rational drug design having boron-containing compounds as main moiety, as well as, the different effects of these compounds on human physiology, particularly on G-Protein coupled receptors. He has authored more than 35 publications that have been cited over 200 times, and he has been serving as an Editorial Board Member and Reviewer of repute scientific journals.

e: soum13mx@gmail.com

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Hepatoprotective activity of aqueous seed extract of *Nigella sativa* against highly active antiretroviral therapy induced hepatotoxicity in rats

Kissi Mudie
Ethiopian Public Health Institute, Ethiopia

Background: Liver is a metabolically active organ responsible for many vital life functions. It performs many activities that are critical for survival. Due to its important activities, the liver is exposed to many insults and is one of the body's organs most subject to injury. Despite tremendous advances in modern medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cell. It is, therefore, necessary to search for effective and safe herbal drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety.

Aim: The aim of this study was to investigate the hepatoprotective activity of aqueous extract of *Nigella sativa* seed in highly active antiretroviral therapy (Lamivudine, Zidovudine and Efavirenz) administered rats.

Materials & Methods: Sixty rats weighed between 150-200 g were randomly divided into six groups and each group comprised of ten rats. Rats in group I were administered with distilled water. Rats in group II were administered with highly active antiretroviral therapy only. Rats in groups III - VI were administered 100, 200, 400 and 800 mg/kg *Nigella sativa* plus highly active antiretroviral therapy respectively. The treatments were given orally for 28 consecutive days. On the 29th day, all rats were sacrificed under light diethyl ether anesthesia; blood samples were collected for the assessment of biochemical parameters, while liver tissue was used for histopathological assessment.

Results: Serum levels of liver enzymes ALT, AST, ALP, and GGT were significantly ($p<0.05$) increased and albumin concentration was significantly decreased in animals treated with highly active antiretroviral therapy as compared to the normal control. Histopathological observations also revealed severe damage in the structure of liver tissue in animals administered with highly active antiretroviral therapy. Treatment of highly active antiretroviral therapy exposed animals with *Nigella sativa* showed marked improvement in both biochemical and histopathological findings. Rise in liver enzymes was almost restored to normal in animals treated with *Nigella sativa.*

Conclusion: *Nigella sativa*, through its antioxidant activity, effectively protects highly active antiretroviral therapy induced liver toxicity.

Speaker Biography

Kissi Mudie has completed his MSc in Medical Biochemistry from Addis Ababa University, School of Medicine. He is the Director of National Clinical Chemistry. Laboratory, Ethiopian Public Health Institute. He has published more than 14 papers in reputed journals and has been serving as an Associate Researcher

e: kissimudiey@yahoo.com

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Novel synthetic inhibitors of eosinophils with potential anti-asthmatic activity

Tarek Aboul-Fadl
Assiut University, Egypt

Asthma is a major public health issue with high and increasing prevalence rates and a concomitant increase in morbidity and mortality. Asthma is estimated to affect 300 million people, with an expected increase to 400 million worldwide by 2025. Many factors may have contributed to the rise of the problem of bronchial asthma. Increasing air pollution, fast modernization, and widespread construction work are some of the reasons for asthma to thrive. The situation is complicated by poor access to medical services and high price of effective drugs.Asthma is a chronic inflammatory condition, triggered by environmental factors in genetically predisposed individuals, and is characterized by mast cell, T lymphocyte, and eosinophil infiltrates in the bronchial mucosa. Eosinophils are recruited to sites of specific inflammatory reactions, especially during allergic diseases and are correlated with asthma severity. In spite of their numerous adverse effects inhaled glucocortocoids have been established as the standard treatment for asthma. Therefore, an urgent need exists for alternative treatments to overcome these undesirable side effects of steroid therapy and to provide another effective agent for the treatment of asthma. Lidocaine was reported to inhibit interleukin-5 (IL-5)-mediated survival and activation of human eosinophils. It can replace inhaled glucocorticoids for the treatment of asthma; however, lidocaine has many undesired side effects mainly due to its sodium channel activity including anesthesia. Accordingly, the current work aims to modify lidocaine structure to obtain analogs with minimum sodium channel and enhanced IL-5 inhibitory activity. The hypothesis supported by ligand-based pharmacophore modeling generated using different molecular modeling programs.

Speaker Biography

Tarek Aboul-Fadl is a Prof. of Medicinal Chemistry at Faculty of Pharmacy, Assiut University/Egypt. Dr Aboul-Fadl received his PhD in Pharmaceutical Medicinal Chemistry from Assiut University (1994) under the channel system and joint supervision scheme between Assiut University and Josai University/Japan. Dr Aboul-Fadl performed his postdoctoral training as a postdoctoral research fellow and Scientist at Institute of Pharmaceutical Chemistry, University of Vienna, Austria (1997- 1998), Institute of Pharmacy and Food Chemistry, University of Erlangen-Nürnberg, Germany (1999 and 2013) and Department of Medicinal Chemistry, University of Utah, USA (2001-2002 and 2004-2005). Dr Aboul-Fadl joined Department of Medicinal Chemistry as an assistant Prof. in 1994, then promoted to associate Prof. in 1999 and to Professor in 2004. Dr Aboul-Fadl is a member of Egyptian Syndicate of Pharmacists since 1984, Egyptian Society of Pharmacists since 1994, American Chemical Society since 2002 and The Stop TB Partnership Working Group on New TB Drugs (WGND) since Feb. 2010

e: fadl@aun.edu.eg

Notes:

Chemical composition, antioxidant and antibacterial activity of *Thuja orientalis* essential oil

Wajaht A Shah and **Mahpara Qadir**
University of Kashmir, India

Essential oils derived from many aromatic plants are well known to possess cytotoxic, antioxidant, antifungal, insecticidal and antimicrobial activities. This work was carried out to evaluate chemical composition, antioxidant and antibacterial activity of *Thuja orientalis* essential oil. *Thuja orientalis* (family: *Cupressaceae*) is widely cultivated as a common ornamental plant. It possesses anti-plasmodial, antioxidant and elastase inhibitory activities. Chemical composition and pharmacological potential of hydro distillate from *Thuja orientalis* are reported in this study. Fresh fruits were subjected to conventional hydrodistillation. Antioxidant activity was assessed as free radical scavenging capacity (RSC) towards 2, 2-diphenyl-1-picrylhydrazil (DPPH) radicals and antibacterial activity was evaluated against six test bacteria by agar well diffusion method. Qualitative and quantitative analysis of *Thuja orientalis* hydrodistillate by gas chromatography coupled with mass spectrometry revealed the presence of nineteen constituents, representing 94.6% of the total oil. The major constituents of oil were alpha-pinene (83%), sabinene (2.6%), delta-3-carene (2.5%). The oil showed appreciable antibacterial effect against all Gram-positive and Gram-negative bacteria tested with MIC values between 12.8-25.6 µg/ml. Therefore this oil could be used in the formulation of antimicrobial and antioxidant agents.

e: shahwajaht@yahoo.com

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Modified glycol chitosan nanocarriers carry hydrophobic materials into tumours

Akhtar Aman
Shaheed Benazir Bhutto University, Pakistan

Development of efficient delivery system for hydrophobic drugs remains a major concern in chemotherapy. The objective of the current study is to develop polymeric drug-delivery system for etoposide from amphiphilic derivatives of glycol chitosan, capable to improve the pharmacokinetics and to reduce the adverse effects of etoposide due to various organic solvents used in commercial formulations for solubilization of etoposide. As a promising carrier, amphiphilic derivatives of glycol chitosan were synthesized by chemical grafting of palmitic acid N-hydroxysuccinimide and quaternization to glycol chitosan backbone. To this end, a 7.9 kDa glycol chitosan was modified by palmitoylation and quaternisation into 13 kDa. Nano sized micelles prepared from this amphiphilic polymerhad the capability to encapsulate up to 3 mg/ml etoposide. The pharmacokinetic results indicated that GCPQ based etoposide formulation transformed the biodistribution pattern. AUC 0.5-24 hr showed statistically significant difference in ETP-GCPQ vs. commercial preparation in liver (25 vs 70, $p<0.001$), spleen (27 vs. 36, $P<0.05$), lungs (42 vs. 136, $p<0.001$), kidneys (25 vs. 30, $p<0.05$) and brain (19 vs. 9, $p<0.001$). Using the hydrophobic fluorescent dyeNile red, we showed that micelles efficiently delivered their payload to MCF7 and A2780 cancer cells in-vitro and to A431 xenografttumor *in-vivo*, suggesting these systems could deliver hydrophobic anti-cancer drugs such as etoposide to tumors. The pharmacokinetic results indicated that the GCPQ micelles transformed the biodistribution pattern and increased etoposide concentration in the brain significantly compared to free drug after intravenous administration. GCPQ based formulations not only reducedside effects associated with current available formulations but also increased their transport through the biological barriers, thus making it a good delivery system.

Speaker Biography

Akhtar Aman has completed his PhD from Peshawar University under Hec Scholarship. During his PhD studies, he also worked as Visiting Scientist at Center for Cancer Medicine, School of Pharmacy, University College London, UK. He is currently serving as Assistant Professor of Pharmaceutics at Shaheed Benazir Bhutto University, Sheringal, Pakistan. He has published more than 10 papers in reputed journals

e: dramanrph@sbbu.edu.pk

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Rational design of guanylthiourea derivatives as antimalarial agents

Shweta Bhagat, Prasad V Bharatam and **Minhajul Arfeen**
National Institute of Pharmaceutical Education and Research, India

Plasmodium falciparum dihydrofolate reductase (P*f*DHFR) enzyme is one of the validated targets for antimalarial drug discovery. The quadruple mutant of P*f*DHFR is resistant to the known anti-P*f*DHFR drugs (e.g. proguanil, pyrimethamine and trimethoprim). Recently, P218 was identified as a potential lead molecule. In this work, a rational drug design strategy was adopted to identify guanylthiourea (GTU) derivatives as a potential P*f*DHFR inhibitor. Electronic structure analysis of the GTU moiety was carried out to determine the correct tautomeric form which was 11.99 kcal/mol more stable than the previously reported structure in the literature. Once acceptable structure was established; *in silico* investigations on the wild type/quadruple mutant P*f*DHFR and various ligands (including MESP analysis, molecular docking studies) were performed to design novel GTU derivatives as potential P*f*DHFR inhibitors. Three series of GTU derivatives were synthesised, by reacting bromides with GTU under reflux and microwave condition. The synthesized compounds were first evaluated for *in vitro* P*f*DHFR inhibitory activity, resulting in the identification of two compounds (100 µM and 0.4 µM). Further, *in vivo* studies recognized six compounds with high mean survival time, out of which one compound was identified to be curative. This work reports a systematic rational approach for the structure-based design of potential antimalarial agents.

e: bhagatshweta61@gmail.com

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Anti-obesity activities of xanthorrhizol in 3T3-L1 adipocytes

Seok Fang Oon
University of Putra Malaysia, Malaysia

According to the World Health Organization (WHO), at least 2.8 million people die every year due to overweight or obesity. The increased obesity prevalence has caused major health problems such as cardiovascular diseases and diabetes. Although several anti-obesity drugs have been developed, they are limited due to adverse side effects such as stroke, myocardial infarction, and depression. These circumstances have increased the demand for effective and safe anti-obesity agents. Previous studies demonstrated that xanthorrhizol (XNT) reduced the levels of serum free fatty acid and triglyceride *in vivo*, but the detailed anti-obesity activities are yet to be reported. Thus, this study aims to evaluate the abilities of XNT to impede adipogenesis, stimulate lipolysis, and its related lipolytic mechanisms employing 3T3-L1 adipocytes. Adipogenesis was examined by glycerol-3-phosphate dehydrogenase (GPDH) activity, whilst lipolysis was investigated by quantifying the glycerol amount. The mechanisms involved were further evaluated by protein analysis of leptin and insulin. Statistical significance was established by one-way ANOVA, where $p<0.05$ and 0.01 were considered statistically significant. In this study, XNT decreased GPDH activity in a dose-dependent manner from 3.125 to 12.5 µg/mL. The highest GPDH inhibition and glycerol release was 47.74±1.36% and 45.37±1.43% ($p<0.05$), respectively. Interestingly, XNT-treated adipocytes produced leptin at levels that were two times higher than control ($p<0.05$) and induced a 64.04±1.73% reduction in insulin expression ($p<0.01$). These results revealed that XNT may suppress adipogenesis and stimulate lipolysis through regulation of leptin and insulin expression in the adipocytes. In this conference, I will discuss the anti-obesity activities of xanthorrhizol and how it works based on our recent protein analysis.

Speaker Biography

Seok Fang Oon has her expertise in investigating the potential health effects of natural products. She is doing her PhD research in the anti-obesity activities of xanthorrhizol in University of Putra Malaysia. She has worked as a Senior Research Officer for two years in the project development of antihypertensive and anti-diabetic natural products. Her main research interests include the biological effects of natural products and herbal treatment in hypertension, obesity, diabetes, hyperlipidermia, and cancer

e: seokfang@live.com

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Video Presentations

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November 02-03, 2017 Chicago, USA

Free radicals production in rat's gastric mucosa during chronic nitrate intoxication

Akimov O Ye
Ukrainian Medical Stomatological Academy, Ukraine

Nitrate intoxication is a serious problem both in Ukraine and in USA. In Ukraine the predominant source of nitrate intoxication is groundwater. That is especially true to regions which are mostly agricultural such as Poltava, Kirovograd, and Nikolayev regions. In USA similar problems arise in Oklahoma, Kansas, Nebraska, Iowa and several other states. Groundwater is not the only source of excessive nitrate intake. Recent trend in creation of nitric oxide (NO) releasing medications can lead to chronic nitrate intoxication in regions where nitrates are abundant in groundwater. Nitrates mostly undergo metabolic changes in gastrointestinal tract. So stomach is one of the first organs to suffer from chronic nitrate intoxication. There are evidences in literature indicating that nitrates can shift NO production. Most scientists tend to blame nitrate-nitrite reduction for the changes. However there is clearly not enough information about functioning of nitrate-nitrite reductases. The superoxide anion radical ($\bullet O_2$-) production also changes during nitrate intoxication. Since •O2- is one of the most common reasons of cell injury and death increase in its production may lead to tissue damage. Functioning of superoxide dismutase (SOD) may also change during nitrate intoxication providing even more risks to tissues.

In this seminar, I will discuss the changes in production of NO and $\bullet O_2$- during chronic nitrate intoxication in gastric mucosa of rats.

Speaker Biography

Akimov Oleh Yeugenovich is a PhD student in HSEEU "Ukrainian medical stomatological academy", department of Pathophysiology. Graduated from the Faculty of Dentistry of the HSEEU "Ukrainian Medical Stomatological Academy" in 2009. Works at the department since 2015. PhD thesis theme: "Mechanisms metabolic disorders in gastric mucosa of rats under conditions of combined excess intake of sodium nitrate and sodium fluoride and their correction by enterosorbents". He is the author of over 20 scientific works and 3 patents for utility models

e: riseofrevan5@gmail.com

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Angiopoietin-like 4 as promoter of angiogenesis and vascular permeability

Silvia Montaner
University of Maryland, USA

Angiopoietin-like 4 *(ANGPTL4)*, a member of the *ANGPTL* family, is involved in many pathological disorders, including cardiac and lung diseases, cancer, retinal diseases, diabetes, atherosclerosis and nephrotic syndrome. This cytokine is a circulating multifunctional protein, which undergoes post-translational modifications (glycosylation) and subsequent proteolytic processing by membrane proprotein convertases, upon secretion of the full-length gene product. ANGPTL4 N-terminal domain *(nANGPTL4)* acts as an adipokine, inhibiting lipoprotein lipase (LPL) and causing hydrolysis of circulating triglycerides (TG) into free fatty acids, under conditions of fasting and exercise. Alternatively, *ANGPTL4* C-terminal domain *(cANGPTL4)* has an important role in anoikis resistance, altered redox regulation, tumorigenesis, and angiogenesis. Compelling evidence suggests a role of *cANGPTL4* in solid tumors, including melanoma, breast carcinoma, hepatocellular carcinoma, renal cell carcinoma and colorectal cancer. The overexpression of *ANGPTL4* in tumors appears to be associated with poor prognosis and poor disease-free survival rates. In our lab, we observed that *ANGPTL4* is a pro-angiogenic factor in Kaposi's sarcoma (KS), a vascular tumor caused by infection with human herpesvirus 8 or KS-associated herpesvirus (HHV-8/KSHV), and a common type of oral cancer in immunocompromised individuals. We observed upregulation of *ANGPTL4* in both oral KS lesions and KS animal models because of the expression of the HHV8/KSHV G protein-coupled receptor (vGPCR), a constitutively-active viral GPCR homolog to CXCR2. The mechanism by which vGPCR induces *ANGPTL4* gene expression includes the activation of Hypoxia Inducible Factor 1 (HIF1). Interestingly, we found that vGPCR-induced *ANGPTL4* upregulation promotes angiogenesis in KS by the potent induction of endothelial cell migration. We also found that *ANGPTL4* promotes vessel hyperpermeability, disrupting both adherens and tight endothelial junctions, an effect that contributes to the profuse edema seen in this tumor. Our results suggest that *ANGPTL4* may be a novel therapeutic target for KS and other disorders associated with pathologic angiogenesis and vascular hyperpermeability.

e: smontaner@umaryland.edu

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