Mukta Asnani Research Advisor: Tatyana Pestova

The mechanism of translation initiation on the genomic RNA of Cadicivirus A: a naturally occurring dicistronic picornavirus

Most eukaryotic mRNAs are translated following cap-dependent initiation, in which ribosomes are first loaded by cap-binding complex which then scan downstream to the initiation codon. During stress when cap-dependent translation is inhibited, an alternative 5'end-independent initiation mechanism is activated. IRES (Internal Ribosomal Entry Site) is cis-acting highly structured element present in 5'UTR of messenger RNA which recruits ribosomes directly onto the mRNA by interacting with canonical initiation factors in non-canonical fashion. These IRESs consist of multiple modules and its activity is the result of combined action of these modules. However, it is still unknown how these modules come together and help ribosome to land on messenger RNA. It also requires specific cellular IRES-trans acting factors (ITAFs), which are thought to maintain the IRES in an active conformation and provide the tissue specificity to the IRES function.

My project is to investigate the translation initiation mechanism of novel 5'UTR IRES of dicistronic picornavirus found in dogs called Cadicivirus A. Our lab is interested in studying this specific IRES because the 3' region of 5'UTR IRES shares 90% similarity with poliovirus 5'UTR IRES. Poliovirus belongs to picornaviridae family which consists of enteroviruses and rhinoviruses. Though poliovirus infection has been eradicated, many enteroviral infections still need special attention. Studying CDV-A IRES will help to understand the translation mechanism of picornavirus family. Using toeprinting techniques we found, similar to poliovirus IRES, initiation factors eIF4G, eIF4A, eIF2, eIF3 and an ITAF called PCBP2 are essential for the translation while eIF4B stimulated the activity of the IRES. In contrast to poliovirus IRES, since the ribosomal loading site is different in both IRESs, eIF1/1A stimulated the activity of CDV-A 5'UTR IRES which suggest base-by-base inspection of domain N during scanning to authentic initiation codon.

Nicholas Chakiryan Research Advisor: Erich Lang

Ureteroenteric Fistula Repair Using Minimally Invasive Radiologic Techniques

Introduction and objective: Ureteroenteric fistula (UEF) is a rare complication of inflammation or trauma that causes a communication between the ureter and a portion of the GI tract. Due to the scarcity of these cases, current literature detailing UEF repair is mostly limited to singular case studies. The primary aim of this study was to investigate the outcome of minimally invasive radiological UEF repair versus open surgical techniques, using a larger population than has been previously documented for this topic.

Methods: We retrospectively reviewed 25 cases of UEF repair from our institutions. Cases were divided into two groups, those treated with minimally invasive radiological techniques (13 cases) and those who underwent open surgical repair (12). We used length of hospital stay and percentage of cases requiring additional intervention as indicators of outcome.

Results: Review of our series revealed a wide variety of UEFs including ureterosigmoid (10 cases), ureteroileal (6), ureterocolic (4), ureteroduodenal (2), ureterofallopian (1), ureteropancreatic (1), and ureteroappendiceal (1) fistulas. Minimally invasive radiological interventions resulted in a significantly shorter length of hospital stay than open surgical repair (5.08 v. 9.75 days; p=0.004). Additionally, comparison of the percentage of cases requiring additional interventions between minimally invasive and open surgical repairs showed no statistical difference (46 v. 50%., p=0.847).

Conclusions: For repair of UEFs, minimally invasive radiological interventions have proven highly successful. Patients treated with minimally invasive techniques had significantly shorter hospital stays than those treated with open surgical repair, with a similar percentage of cases requiring additional interventions. These techniques should be considered the standard of care for the initial treatment of UEFs.

Subhash Chandra Research Advisor: Chinmoy Gulrajani

Is It Time For a Federally Mandated Assisted Outpatient Treatment Program?

Forty Five U.S. states authorize some form of AOT (assisted outpatient treatment) program.

In New York AOT has been shown to reduce re-incarceration, re-hospitalization and homelessness. Involuntary outpatient commitment laws are state specific, serious and persistent mentally ill (SPMI) individuals under state mandated AOT can lose care if they choose to leave the state, leading to a decline in their overall functioning.

45 U.S. states authorize some form of AOT (assisted outpatient treatment) program. In New York AOT has been shown to reduce re-incarceration, re-hospitalization and homelessness. Involuntary outpatient commitment laws are state specific, serious and persistent mentally ill (SPMI) individuals under state mandated AOT can lose care if they choose to leave the state, leading to a decline in their overall functioning. This study was conducted to look in to the advantages of having a federally mandated AOT program with a unified policy across the nation. Review of AOT statutes of all 50 states of the US was done; similarities and differences between these statutes were compared and contrasted. The definition of mental illness, grave disability and the criteria for mandating AOT in different states shows a wide range of variation. Federal mental health court program is an innovative and successful approach in diverting offenders into treatment programs and easing the burden on criminal justice system. Similarly with a federally run AOT program there will be a co-ordinated delivery of services across states with no lapse in the treatment thus decreasing the overall cost and providing continuity of mental health care across states.

Olipriya Das Research Advisor: Richard Kollmar

Otolin 1 and the Ultrastructure of Vertebrate Ear Stones

Otoliths ("ear stones") in fishes and the homologous otoconia in higher vertebrates are acellular biominerals and essential for the sense of balance. Dislodged otoconia inside the inner ear cause benign paroxysmal positional vertigo, the most common form of vertigo in humans. An important component of mammalian and piscine otoliths is the protein otolin 1. It contains both a collagen and a C1q domain, similar to the atypical collagens VIII and X found in Descemet's membrane of the cornea and in developing cartilage, respectively. We propose a model whereby otolin 1 forms a scaffold or "framework" to which other otolith proteins as well as calcium and carbonate ions bind during otolith morphogenesis. According to this model, the two domains of otolin 1 are critical for framework formation: trimers of C1q domains form its hubs, and triple-helices of collagen domains form its spokes. By analogy, we propose that the C1q domain of otolins directs the assembly of protein trimers that are required to form the extracellular matrix of otoliths and otoconia. Here, we will test this model by creating mutant forms of zebrafish otolin 1a protein and test whether the C1q domain is necessary and sufficient for trimer formation in vitro by biochemical means. Also a recombinant otolin 1 C1q domain fused to Thioredoxin, a protein that normally exists as a monomer, is being bacterially expressed and will examine whether the C1q domain of otolin 1 is capable of driving the trimerization of thioredoxin. Furthermore, we have raised polyclonal antibodies against otolin 1 as a reagent for binding and expression studies. Trimerization will be assessed by polyacrylamide gel electrophoresis and size exclusion chromatography. Our studies will help to elucidate the molecular basis of otolith ultrastructure, an important step towards understanding how otoliths are formed and maintained in the healthy and the ailing ear.

Julia Jaffe Research Advisor: Michele Haughton

The Quality of Web-Based Information on Advance Directives

Objective: To determine the quality of information that patients encounter in a websearch regarding advance directives. **Methods**: Four search engines (Google, Yahoo, Bing, Ask) were queried for search terms, "advance directives," "health care proxy," "DNR/DNI," and, "living will." The websites on the first page of each search engine were used for analysis. Paid ads were excluded. Websites were characterized as non-profit (NPR), government (GOV), university related (UNI), commercial (COM), or reference (REF). Websites were evaluated using the DISCERN Instrument, a 16 question validated tool designed to assess quality based on aims, relevance, references, treatment options, and overall impression. Results: 134 unique sites were identified. Of these, 58 were paid advertisements, and 76 were evaluated. The mean DISCERN Score was 40.0 +/-16.4 (poor quality). 40 were of poor quality (<44), 32 of average quality (44-67), and 4 of excellent quality (>56). 35 were commercial sites, 21 were nonprofit or personal sites, 10 were government sponsored sites, 5 were University sites, and 6 were reference sites. There was a trend toward higher scores for sites characterized as University-related, Reference, and Non-profit (55.0 +/-3.4, 49.7 +/- 11.1, and 47.9 +/- 16.3, respectively) than for Government and Commercial sites (40.3 +/- 9.7 and 31.2 +/-14.9, respectively). There were no statistically different differences among sites by search term or search engine used. Conclusions: When patients and families search the internet for information regarding advance directives, the majority of websites encountered are average to poor quality. Physicians should discuss with their patients end-of-life care, guide patients in internet searches for related information, and discuss the limitations of online resources.

Cliff Kerr Research Advisor: William Lytton

Network-Level Effects of Optogenetic Stimulation: Experiment and Simulation

Only small numbers of neurons can be typically be recorded from during optogenetic perturbation. To address this limitation, we present a model of motor cortex (M1) to explore the network-level effects of optogenetics. Experimental data were recorded from two macaques. The model consisted of 24,800 spiking neurons, with network parameters and opsin properties drawn from empirical literature. The model was calibrated to reproduce observed firing rates and dynamics. Experimentally, optogenetic stimulation was found to increase firing rates by up to 300 spikes/sec, with higher firing closer to the optrode; firing returned to baseline levels 4-5 mm away. A similar response was observed in the model. Manipulating the strength of synaptic connectivity in the model elucidated several noteworthy aspects of the response. First, the decrease in peak firing rates very near the optrode is due to recruitment of inhibitory neurons. Second, heterogeneities in connectivity were sufficient to account for the broad range of observed firing rates. Third, all of the high-firing excitatory cells (>100 spikes/sec) were directly opsin-expressing. Applying spectral Granger causality to the local field potentials in the model showed that the strongest projection was from cortical layer 2/3 to layer 5A, consistent with descending excitation being the primary driver of dynamics in M1. Strong causality from layer 5A to layer 5B was also observed. Granger causality showed a pronounced peak in the mu rhythm band (~9 Hz); a small bump in the gamma band (~40 Hz) was also observed in pathways from layer 2/3 to other layers. Optogenetic stimulation increased the amplitude of causality from layer 2/3 to other layers in the mu rhythm band, while decreasing it in the gamma band. In summary, this work demonstrates that (1) synaptic connections determine the network response to optogenetic stimulation, and (2) optogenetic stimulation may be used to modulate information flow in particular frequency bands.

Francisco Mendoza Jr. Research Advisor: Meriam Caboral-Stevens

Length of intubation between intravenous acetaminophen versus opioids in older adult's post-transcatheter aortic valve implantation (TAVI)

Introduction: With the growth in life expectancy for the generations to come, there is a higher prevalence for valvular disease. Advances in cardiac surgeries have provided alternative options for elder adults who were not candidates for open-heart surgery before. Further, the advent of these innovative advances in cardiac surgeries such as TAVI, are expected to exceed the projected recovery, functionality, and quality of life. Using intravenous (IV) acetaminophen for this population may decrease opioid requirement, thus decrease their length of intubation.
Method: Guided by Betty Neuman's Open Systems Theory, this quantitative, prospective, and double-blind experimental study design is to test the hypothesis that in elderly adults undergoing TAVI, the administration of IV acetaminophen in conjunction with opioids (Group AF) will decrease the time of intubation post-operatively compared to those who receive IV opioids alone (Group F).Evenly randomized into two groups, 24 participants will be used, in order to obtain a power of 90%, a large effect size of 1.1 and significance level of 0.05 to test the hypothesis. Participants include males and females, 70 years and older with American Society of Anesthesiology (ASA) Class III-IV. Group AF will receive IV acetaminophen 1gm every 6hrs mixed in 100 ml solution and IV fentanyl, while those in Group F will receive 100 ml of IV saline solution every 6hrs and IV fentanyl. Descriptive statistics and Mann-Whitney tests will be the statistical analyses used to test the hypothesis.
Summary: This study will exemplify delivery of optimal patient care as well as clinical outcome, particularly in the elderly population. This research project will also quantitatively assess the dynamics associated with interventional airway management perioperatively.

Peiqi Ou Research Advisor: Chongmin Huan

Identification of Sphingomyelin Synthase 2 as a PKCd Activator That Mediates Peripheral B cell Tolerance

B cell tolerance prevents the development of B cell-mediated autoimmunity. PKCd is an essential mediator of B cell tolerance since it is required for the deletion of central and peripheral autoreactive B cells via different mechanisms. Although a recent study has characterized the role of PKCd in Ca2+ signaling-controlled central B cell tolerance, the regulation of PKCd nuclear translocation that eliminates peripheral autoreactive B cells is largely unknown.

Sphingomyelin synthase (SMS) catalyzes the synthesis of sphingomyelin while generating diacylglycerol (DAG) as a by-product. The potential role of SMS as a DAG provider for activation of PKC family proteins was proposed decades ago, but neither the physiological targets nor the functional significance of SMS-derived DAG has been determined.

Mammalian cells have two major SMS isoforms. SMS1 is localized in the ER and Golgi membranes while SMS2 is found in the plasma membrane. In this study, we found that SMS2-/- mice, but not SMS1-/- mice, had a B cell autoimmune phenotype resembling the phenotype of PKCd-/- mice. Further analysis showed SMS2-/- B cells had reduced DAG levels and impaired PKCd nuclear translocation that could be partially rescued by adding a DAG analog. Consistently, we found that SMS2 physically interacted with PKCd, but not other PKCs in primary B cells. These results indicate that SMS2 is a novel PKCd activator that mediates peripheral B cell tolerance.

Autoimmune diseases remain a major clinical challenge due to the limited knowledge of their complicated pathogenesis. While genetic PKCd deficiency has been recently identified as a cause of impaired B cell tolerance in autoimmune patients, our study suggests that dysregulated SMS2-mediated PKCd function could lead to human autoimmunity as well, thus providing insight into the development of new diagnostic and therapeutic approaches.

Puja Shah Research Advisor: Roulhac Darby Toledano

Left Lower Extremity Weakness after Vaginal Delivery and Combined Spinal Epidural Anesthesia

A 24-year old G3P2 presented to our L&D unit at term gestation for elective induction of labor. She had a hx of two previous nsvd without complication. She was otherwise healthy, with no prior surgical or medical history On PE, she had a thin body habitus, a MP class I airway, and normal physical exam.At 3 cm cervical dilation the patient requested labor analgesia, and a CSE was placed at L3-4 on first attempt. A wire-reinforced catheter threaded with ease 6 cm into the epidural space with no signs of intravascular or intrathecal placement An epidural infusion of 0.1% bupivacaine with 2mcg/mL of fentanyl was initiated. The patient remained comfortable for the next 5 hours, until she reached 5 cm of cervical dilation. At that point she requested a top-up primarily due to perineal pain. The resident performed a test dose with 1.5% lidocaine with epinephrine followed by a manual bolus of 4 ml of the bupivacaine/fentanyl infusate. Upon receiving the manual bolus the patient immediately felt numbness from the mid-back to her lower extremities and complete loss of motor function of the lower extremities. Perineal pain, however, persisted.Her labor progressed rapidly from that point, but she was unable to push or participate in the second stage of delivery. Two assistants held her legs and fundal pressure was applied. Motor and sensory deficits persisted postpartum, and a lumbar MRI was performed to rule out epidural hematoma. Over the next 12 hours, the patient experienced complete return of her RLE motor strength, but persistent weakness in her left lower extremity. Although the incidence of migration of single end-hole, wire-reinforced catheters in the setting of an uneventful CSE is reported to be rare, two neurologists independently determined that this patient experienced a hemicord lesion due to either intrathecal migration of the catheter during the manual injection of bolus or direct mechanical injury from the catheter on the spinal cord.

Meghan Walsh Research Advisor: Mahmood Hussain

A Novel Mutation in MTP Highlights the Importance of the N-terminal β-sheet in its Lipid Transfer and ApoB Secretion Activities

Microsomal triglyceride transfer protein (MTP) is critical for the assembly and secretion of apolipoprotein B (apoB)-containing lipoproteins. Mutations in the MTTP gene cause abetalipoproteinemia (ABL). Here, we identified a homozygous missense mutation in the MTTP gene in a 4-month old Turkish male. He presented at the Ankara Diskapi Children's Hospital with classical ABL symptoms. HDL-cholesterol and apoA1 levels were normal (41 and 110 mg/dL, respectively), but total cholesterol was low (47 mg/dL). Plasma triglyceride (TG), LDL-cholesterol, and apoB were undetectable. Sequencing revealed a mutation at amino acid 169 (MTPD169V) located in the N-terminal β-sheet of MTP. Previously characterized ABL missense mutations were in the central α-helical and C-terminal β-sheet domains and highlighted the importance of these domains for the lipid transfer activity of MTP. The N-terminal domain, conversely, has mainly been implicated in apoB- and membrane-binding. Although MTPD169V was present in the endoplasmic reticulum and partially co-localized with PDI, it was unable to transfer TG and phospholipids (PL). Further, MTPD169V did not support the assembly and secretion of apoB-containing lipoproteins. Computational molecular modeling suggested that D169 may form an internal salt bridge with K187 and K189. Indeed, mutagenesis of these lysines to leucines abolished the TG transfer, PL transfer, and apoB secretion activities of MTP. Further, conservative mutagenesis that preserved charges at residues 169, 187 and 189 partially restored these activities. Thus, D169 is likely involved in an internal salt bridge with K187 and K189. Disruption of this internal salt bridge in the N-terminal region abolishes the lipid transfer activities present in the C-terminal end of the MTP molecule. We speculate that this salt bridge, although away from the proposed lipid transfer site, might be important in providing structural integrity necessary for the lipid transfer activity of MTP.