2nd Annual Molecular Pathobiology Innovative Research Symposium

June 10, 2022
New York University College of Dentistry

Location: 433 1ST AVE, ROOMS 220 & 210
Zoom: https://nyu.zoom.us/j/98901390433

Program

Time	Session	Speaker / Title
8:50-9:00	Welcome & Opening remarks (Rooms: 220 & 210)	**Brian L. Schmidt, DDS, MD, PhD** (Vice Dean for Research and Faculty Affairs, NYU College of Dentistry)
9:00-09:50	**Oral Session I: Cell signaling- Pain/ Cancer** (Chair: Alan Hegron) Rooms: 220 & 210	**Kimberly Gomez** / Harnessing a unique NaV1.7 regulatory domain for chronic pain
		Maria Daniela Santi / Exploring Schwann cell L1CAM-EGFR interaction in oral cancer pain
		Santiago Loya-López / The natural product argentatin C attenuates postoperative pain via inhibition of voltage-gated sodium and T-type voltage-gated calcium channels
09:50-10:05	BREAK	
10:05-10:55	**Oral Session II: Cell signaling- Inflammation/ Immunology** (Chair: Kritika Srinivasan Rajsri) Rooms: 220 & 210	**Sally Morris** / ANT participation in Mitochondrial Permeability Transition Depends on the Induction mechanism
		Tamires Duarte Afonso Serdan / Axon guidance molecule Slit3 is essential for brown adipose tissue thermogenesis
		Fangxi Xu / E-cigarette use promotes a smoking-alike salivary microbiome in periodontitis
10:55-11:15	**Oral Session III: Education** (Chair: Min Young Park) Rooms: 220 & 210	**Johanna Warshaw** / Helping students succeed on the Integrated National Board Dental Exam
11:15-11:30	BREAK	
11:30-12:15	**Keynote lecture** (Chair: Min Young Park) Rooms: 220 & 210	**Stavroula Kousteni, PhD** (Columbia University Medical Center, Department of Physiology and Cellular Biophysics) / Title: The osteoblastic niche in Acute Myeloid Leukemia progression, prevention and treatment
12:15-1:00	**BREAK/ LUNCH** 433 1st Ave Courtyard	

1:00-1:35	**Poster Session IA: Room - A**	**P-1. Divya Bhansali** / Tackling PAR-mediated Cancer Pain with Nanocarriers **P-2. Kritika Srinivasan Rajsri** / An Immunity Screen Assessing Covid-19 And Vaccination Induced Antibody Response At Point-Of-Care **P-3. Rachel Pollard** / Highly Tunable Fluorescent Nanoparticle Library for Optimizing Neuronal and Gastrointestinal Drug Delivery **P-4. Vasudev Vivekanand Nayak** / Physiochemical and bactericidal activity evaluation: Silver-augmented 3D-printed scaffolds - An *in vitro* study **P-5. Gretchen Cerezal** / Effects of LED Curing Light on Silver Diamine Fluoride Penetration into Dentin **P-6. Lillian Moran** / Facilitating Faculty Collaboration and Integration of Didactic and Clinical Curriculum.
	Poster session IB: Room - B	**P-7. Casey Griffin** / SF3B2 knockdown disrupts neural crest cell development in *Xenopus* **P-8. Lenny J Negrón-Piñeiro** / *Cis*-regulatory control of stage-specific notochord gene expression by Brachyury **P-9. Prem Prakash** / Role of SUCNR1 Signaling in Myeloid Lineage Homeostasis **P-10. Carole Le Henaff** / Abaloparatide at the same dose has the same effects on bone as PTH (1-34) in mice **P-11. Min Young Park** / Adipocyte-specific deletion of FGF23 alleviates diet-induced obesity in mice **P-12. Sher Bahadur Poudel** / Long term exposure to methylene blue treatment in aged mice causes trabecular bone loss
1:35-2:10	**Poster session IIA**: **Room - A**	**P-13. Alan Hegron** / PAR2 in chronic pain: From physiology to molecular characterization to structural study **P-14. Ammar Khan** / Pharmacological Inhibition of CGRP Receptor Reduces Cancer Nociception **P-15. Ga-Yeon Son** / SOCE regulates MMP-1 expression modulating the transformation of dysplasia to oral cancer **P-16. Maria A. Neginskaya** / ATP synthase is essential for the high-conductance mitochondrial permeability transition but not for stress-induced mitochondrial depolarization **P-17. Morgan Zhang** / ELB00824, a novel PPAR gamma agonist, prevents oxaliplatin-induced pain and reduces spinal oxidative stress

	Poster session IIB: **Room - B**	**P-18. Chloe J. Peach** / Modulation of the spatiotemporal dynamics of NGF-mediated signaling **P-19. Paz Duran** / Identification of a novel neuropilin 1 inhibitor that blocks CRMP2 phosphorylation and reverses mechanical allodynia and thermal hyperalgesia in a rodent model of neuropathic pain **P-20. Yaw A. Akosah** / mPTP induction by polyP is chain length-dependent **P-21. Aida Calderon-Rivera** / Pentacyclic triterpenoids inhibit N- and T-type voltage-gated calcium channels to attenuate nerve-injury associated neuropathic pain **P-22. Lin Y. Hung /** Serotonin Of The Intestinal Mucosa Alleviates Anxiety And Depression Independently Of Gastrointestinal Function And The Ens **P-23**. **Sarah Najjar** / Early life adversity alters enteric nervous system development, gut motility, and visceral sensitivity
2:10-2:25	BREAK	
2:25-3:15	**Oral Session IV: Biomaterials** (Chair: Sasan Rabieh) Rooms: 220 & 210	**Andrea Shill** / Deposition of Zinc Phosphate Solutions on Carious Dentin *in Vitro*
		Gokul Sriman Thanigai Arasu / Nanoparticle-based, targeted delivery of PAR-2 antagonist in an *in vivo* inflammatory pain model
		Parker K. Lewis / Slow-Release Nanoparticles for Chronic Pain Management via Intrathecal Delivery
3:15-3:35	BREAK	
3:35-4:25	**Oral Session V: Skeletal, Craniofacial & Developmental Biology** (Chair: Nadege Gouignard) Rooms: 220 & 210	**Arun Devotta** / The core splicing factors EFTUD2, SNRPB and TXNL4A are essential for neural crest and craniofacial development
		Guilherme H. Souza Bomfim / Ca^{2+} clearance Mediated by Pumps (PMCAs) and Exchangers (NCKXs) in Enamel Cells
		Nadege Gouignard / MMP28 triggers EMT by regulating *twist* and *cadherin-11* expression in neural crest cells
4:25-4:45	BREAK	
4:45-5:15	Prizes & Concluding remarks Rooms: 220 & 210	**Nigel W. Bunnett, PhD** (Chair, Department of Molecular Pathobiology, NYU College of Dentistry)
5:15-7:00	**HAPPY HOUR** 433 1st Ave Courtyard	

11:30 AM-12:15 PM
Keynote lecture

Rooms: 220 & 210

Keynote Speaker: Stavroula Kousteni, PhD

(Columbia University Medical Center, Department of Physiology and Cellular Biophysics)

The osteoblastic niche in Acute Myeloid Leukemia progression, prevention and treatment

Stavroula Kousteni, Ph.D. is the Edward P. Evans Professor in Physiology & Cellular Biophysics and the Herbert Irving Comprehensive Cancer Center, the Director of the Edward P. Evans Center for Myelodysplastic Syndromes at Columbia University and a member of the Stem Cell Initiative at Columbia University. She joined Columbia University as an Assistant Professor in 2006. Prior to that she was an Assistant Professor of Medicine at the University of Arkansas for Medical Sciences, Little Rock (1999-2006). She received her Ph.D. degree from the University of Wales (1990-1994), followed by postdoctoral training at the School of Molecular and Medical Biosciences in Wales (1994-1997), UK, and the Hellenic Pasteur Institute in Athens, Greece (1997-1999).

The aim of Dr. Kousteni's laboratory is to examine the role of the bone marrow microenvironment (or niche) in hematopoietic stem cell fate and function, in particular during the development of: Myelodysplasia (MDS) and Acute Myeloid Leukemia (AML). Her work has shown that a mutation in osteoblasts enables in mice MDS rapidly progressing to AML. This pathway can sustain MDS development and progression to AML in a subset of patients with MDS, since it is active in these patients' osteoblasts. The finding that a stromal cell can directly induce MDS/AML is paradigm shifting and enhances the potential for drug therapy since the osteoblast in MDS or AML may be more amenable to drug therapy than the HSCs since it lacks the propensity to mutate and clonally expand. Dr. Kousteni has followed up this work with projects aiming to identify HSC intrinsic and extrinsic mechanisms, the latter induced by mesenchymal stem cells, that promote transformation of MDS to AML, identifying the subpopulations in the bone marrow microenvironment and the mechanisms through which they interact with malignant cells to regulate disease progression. We are delineating molecular pathways that induce or select for genetic alterations in pre-malignant hematopoietic stem cells that transform them to malignant ones and the specific stem cell functions that they are affecting. We apply cutting edge genetic tools, and single cell technology to integrate studies of clonal mutational evolution and niche components in patients and mouse models, to establish the mechanisms through which the aging marrow supports the outgrowth of AML initiating clones. The ultimate goal of our work is to identity therapies that target oncogenic signals stemming from the bone marrow microenvironment to prevent MDS and AML transformation and overcome targeted or standard of care therapy resistance.

Dr. Kousteni served in the Council of the American Society for Bone and Mineral Research (ASBMR), as the basic science Chair of the ASBMR 2020 Annual Meeting Program Committee, the Endocrine Society Annual meeting Steering Committee; an ASBMR Task force to Expand Musculoskeletal Research, the ASBMR Program Committee, the Advisory board of the ASBMR Education Resource Center. In CUIMC she serves at the Faculty Council of the Faculty of Medicine, the Dean's Executive Committee of the Faculty Council, and the Dean's Advisory Committee for Women Faculty. She is the Director of a T32 in Endocrinology and Metabolism: "Hormones: Biochemistry and Molecular Biology". She is at the editorial board of JBMR and Bone research and an associate editor of JBMR Plus.

Honors/Awards: Ph.D. Fellowship The Schilizzi Foundation, Surrey, UK,1991 – 1994; BBSRC (Biotechnology and Biological Science Research Council) postdoctoral fellowship UWC, UK. 1994 – 1997; Research Career Award from The General Secretariat of Research and Technology, The Greek Department of Industry and Development 1997 – 1998; ASBMR Travel Award, 2000; Charles W. Bohmfalk Prize, Columbia University a distinction that recognizes significant contributions to medical science and education, 2008; Irma T. Hirschl Research Award for highly meritorious medical research conducted at Columbia University, 2009; Fuller Albright Award: in recognition of outstanding scientific accomplishments in the bone and mineral field, 2013; Outstanding basic science award of the ASBMR, 2013.

9:00-9:50 AM
Oral Session I: Cell signaling-Pain/ Cancer

Rooms: 220 & 210

Oral Presentation 1

Harnessing a unique NaV1.7 regulatory domain for chronic pain.

Kimberly Gomez[1], Harrison J. Stratton[2], Paz Duran[1], Dongzhi Ran[2], Lisa Boinon[2], Santiago Loya[1], Cheng Tang[1], Aida Calderon-Rivera[1], Liberty François-Moutal[3], Aubin Moutal[3], Cynthia L. Madura[2], Shizhen Luo[2], Samantha Perez-Miller[1], May Khanna[1], and Rajesh Khanna[1,2]*

[1]Department of Molecular Pathobiology, College of Dentistry, New York University, New York, NY, 10010, USA.
[2]Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, AZ, 85724 USA.
[3]Department of Pharmacology and Physiology, School of Medicine, St. Louis University, St. Louis, MO, 63104, USA.

Background: Treatment of chronic pain remains a major challenge for modern medicine and few treatments are both safe and effective.

Hypothesis: Here we test the hypothesis that a rationally designed NaV1.7-derived peptide can disrupt collapsin response mediator protein 2 (CRMP2)-NaV1.7 coupling.

Methods: Using a peptide microarray, we report the discovery of a 15 amino acid regulatory sequence unique to NaV1.7 that is essential for its function.

Results: We show that this unique domain serves as the binding interface for a crucial NaV1.7 regulatory protein, the cytosolic phosphoprotein CRMP2, and that binding between this domain and CRMP2 is critical for neuropathic pain. Using microscale thermophoresis, we found CRMP2 bound to this peptide with low micromolar affinity (0.99 µM). Importantly, when this region is lost, tetrodotoxin sensitive currents in sensory neurons are approximately halved without affecting tetrodotoxin resistant currents. Furthermore, treatment with a membrane delimited cell penetrant interfering peptide (Myr-TAT-NaV1.7), reduced currents through NaV1.7 channels in rat sensory neurons and reversed mechanical allodynia in a rat spared nerve injury model of neuropathic pain. We demonstrate that this peptide exerts its effects by altering NaV1.7 trafficking, resulting in decreased presynaptic NaV1.7 localization and therefore reduced spinal CGRP release. Interfering with NaV1.7-CRMP2 coupling does not result in unwanted side effects, such as motor impairment, and spares thermal, inflammatory, and post-surgical nociception. Finally, we reveal that AAV mediated delivery of the peptide encoding sequence reduces currents through NaV1.7 channels and relieves mechanical allodynia.

Conclusion: These data provide proof of concept for NaV1.7 targeted gene therapy.

9:00-9:50 AM
Oral Session I: Cell signaling-Pain/ Cancer

Rooms: 220 & 210

Oral Presentation 2

Exploring Schwann cell L1CAM-EGFR interaction in oral cancer pain

Maria Daniela Santi, Morgan Zhang, Yi Ye

Backgrounds: Oral squamous cell carcinoma (OSCC) is painful and the molecular mechanism of OSCC pain is poorly understood. L1 Cell Adhesion Molecule (L1CAM) is implicated in cell migration, adhesion, neurite outgrowth, myelination and neuronal differentiation by promoting homophilic and heterophilic interactions. L1CAM is expressed on Schwann cells (SCs) and its overexpression conduces to cancer progression, invasion, and metastasis. It was reported that L1CAM interacts and activates the Epidermal Growth Factor Receptor (EGFR), a transmembrane protein with key roles in the development and progression of many cancers including OSCC. Both L1CAM and EGFR have been recently implicated as regulators of chronic pain.
Hypothesis: Overexpression of L1CAM in SCs activates EGFR to promote oral cancer pain.

Methods: SCs at 70% confluency were treated with L1CAM (5 µg/mL), AG1478 (200nM, EGFR inhibitor) or vehicle. After two days, cells were stained to evaluate immunofluorescence EGFR expression, and supernatants were collected for nociceptive behavior measurements. The mid-plantar right hind paw of C57BL/6J female mouse was injected with SCs supernatant for 4 consecutive days and mechanical threshold was determined 1 h after injection using von Frey filament.

Results: We observed that L1CAM increased the *in vitro* expression of EGFR in SCs (64.8 ± 15.4%) over the control. L1CAM treated SCs supernatant tended to decrease mechanical thresholds from baseline (15.19 ± 4.9%), which were increased by AG1478 treatment.

Conclusions: Our preliminary results show that L1CAM induces EGFR expression in SCs and L1CAM may result in mechanical allodynia in an EGFR dependent manner.

9:00-9:50 AM
Oral Session I: Cell signaling-Pain/ Cancer

Rooms: 220 & 210

Oral Presentation 3

The natural product argentatin C attenuates postoperative pain via inhibition of voltage-gated sodium and T-type voltage-gated calcium channels

Santiago Loya-López[1,a], Paz Duran[1,a], Dongzhi Ran[2], Cheng Tang[1,3], Aida Calderon-Rivera[1], Kimberly Gomez[1], Harrison Stratton[2], Ya-ming Xu[4], E. M. Kithsiri Wijeratne[4], Samantha Perez-Miller[1], Zhiming Shan[2], Song Cai[2], Anna Therese Gabrielsen[2], Angie Dorame[2], Kyleigh Ann Masterson[2], Omar Alsbiei[2], Cynthia L. Madura[2], Guoqin Luo[3], Aubin Moutal[2], John Streicher[2], A. A. Leslie Gunatilaka[4], Rajesh Khanna[1, 5]*

[1]Department of Molecular Pathobiology, College of Dentistry, New York University, New York, New York, United States of America
[2]Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, Arizona 85724, United States of America
[3]Dept. of Biochemistry & Molecular biology, College of life sciences, Hunan Normal University, Lushan Road, No. 36, Yuelu District, Changsha, Hunan Province, China
[4]Southwest Center for Natural Products Research, School of Natural Resources & the Environment, College of Agriculture & Life Sciences, The University of Arizona, Tucson, AZ, 85706, United States of America
[5]Comprehensive Pain and Addiction Center, The University of Arizona, Tucson, Arizona, 85724, United States of America

[a]These authors contributed equally to this work.

Background: Postoperative pain occurs in as many as 70% of the over 230 million surgeries performed annually worldwide. Its management still relies on opioids despite their negative consequences, resulting in a public health crisis. Therefore, it is of utmost importance to develop alternative therapies to treat chronic pain. Natural products derived from medicinal plants are potential sources of novel biologically active compounds for development of safe analgesics.
Hypothesis: Argentatin-C may represent a novel therapeutic approach to treat painful conditions.

Methods: We used ratiometric calcium imaging, whole-cell electrophysiology, in silico docking, and in vivo pain modeling to evaluate a natural product library.

Results: We screened a library of natural products to identify small molecules that target the activity of voltage-gated sodium and calcium channels due to their important roles in nociceptive sensory processing. We found that fractions derived from Parthenium incanum, inhibited depolarization-evoked calcium influx in rat DRG neurons. Further separation of these fractions yielded a cycloartane-type triterpene identified as argentatin C which blocked the activity of both voltage-gated sodium and calcium channels in calcium imaging assays. Docking analysis predicted that argentatin C may bind to NaV1.7-1.9 and CaV3.1-3.3 channels. Furthermore, electrophysiological experiments showed that argentatin C decreased Na+ and T-type Ca2+ currents as well as excitability in rat and macaque DRG neurons. Consistent with these observations, argentatin C treatment reversed mechanical allodynia in a mouse model of postsurgical pain.

Conclusions: The dual role of argentatin C on voltage-gated sodium and calcium channels supports its potential as a novel treatment for painful conditions.

10:05-10:55 AM

Oral Session II: Cell signaling-Inflammation / Immunology

Rooms: 220 & 210

Oral Presentation 4

ANT participation in Mitochondrial Permeability Transition Depends on the Induction mechanism

Sally Morris, Maria Neginskaya, Evgany Pavlov

Mitochondrial Permeability Transition (PT) refers to the phenomenon of stress-induced increase in permeability of the inner mitochondrial membrane, caused by the opening of the nonspecific channel PT pore. Adenine Nucleotide Translocator (ANT) is an important component of PT, however, its precise role remains controversial.

We hypothesized that ANT involvement in PT might depend on the specific pathway of PT activation. We utilized holographic and fluorescent microscopy to directly assess the contribution of ANT towards PT in living cells. Calcium stress was induced by two calcium ionophores, ionomycin and ferutinin. Ferutinin induces PT by electrogenically transporting calcium across the mitochondrial membrane. Ionomycin stimulates PT by causing an increase in cytosolic calcium, followed by mitochondrial calcium uptake. We found that in MEF WT cells, calcium stress induced by ferutinin caused CSA-sensitive depolarization of the inner mitochondrial membrane followed by PT. In cells lacking ANT, we observed only CSA-sensitive membrane depolarization, but not high-conductance PT. Calcium stress induced by ionomycin resulted in depolarization that was not CSA sensitive and was followed by mitochondrial swelling but not high conductance PT in MEF WT cells. Lack of ANT resulted in a delay in mitochondrial depolarization.

We **conclude** that the role of ANT in PT depends on the specific method of calcium-induced mitochondrial stress. In conditions of direct mitochondrial calcium overload, ANT is required for high conductance PT but not depolarization. ANT contributes to mitochondrial depolarization in conditions of global calcium overload.

10:05-10:55 AM

Oral Session II: Cell signaling-Inflammation / Immunology

Rooms: 220 & 210

Oral Presentation 5

Axon guidance molecule Slit3 is essential for brown adipose tissue thermogenesis

Tamires Duarte Afonso Serdan[1], Heidi Cervantes[1], Farnaz Shamsi[1,2]

[1]Department of Molecular Pathobiology, New York University College of Dentistry, New York, NY 10010
[2]Department of Cell Biology, New York University School of Medicine, New York, NY, 10010, New York, NY 10010

Background: Brown adipose tissue (BAT) is a specialized type of adipose that is primarily responsible for thermoregulation. Brown adipocytes have an exceptional ability to oxidize fuels and generate heat to maintain euthermia in a cold environment. Cold exposure enhances BAT thermogenesis through the coordinated induction of brown adipogenesis, angiogenesis, and sympathetic innervation. However, little is known about how these distinct processes are spatiotemporally coordinated. We have recently used single cell RNA-sequencing data to infer the intercellular communications mediated by ligand-receptor complexes in BAT. We identified Slit guidance ligand 3 (Slit3) and its receptor, roundabout guidance receptor 4 (Robo4), as a potential crosstalk axis between adipocyte progenitors, vascular endothelial cells, and sympathetic neurites.

Hypothesis: We hypothesized that Slit3 stimulates endothelial cells and sympathetic nerves through Robo4 and Robo1 to promote angiogenesis and neurite growth, respectively.

Methods: We used adeno-associated virus (AAV)-mediated Slit3 shRNA delivery to reduce Slit3 expression in BAT.

Results: Loss of Slit3 impaired BAT thermogenesis indicated by the reduced ability of mice to maintain their body temperature in cold. Importantly, loss of Slit3 reduced both vascular and sympathetic neurite density in BAT. Conversely, the overexpression of Slit3 in BAT enhanced BAT thermogenesis.

Conclusion: Our results, for the first, demonstrate the role of axon guidance molecules in adipose tissue remodeling and identify Slit3 as a key regulator of adipose tissue thermogenesis. By identifying a new pathway involved in the physiological remodeling of adipose tissue, these findings introduced a potential node of intervention to combat obesity and metabolic disease.

10:05-10:55 AM

Oral Session II: Cell signaling-Inflammation / Immunology

Rooms: 220 & 210

Oral Presentation 6

E-cigarette use promotes a smoking-alike salivary microbiome in periodontitis

Fangxi Xu[1], Scott C. Thomas[1], Mridula Vardhan[1], Angela R. Kamer[2], Xin Li[1], Deepak Saxena[1]

[1]Department of Molecular Pathobiology, New York University College of Dentistry, New York, NY, United States.

[2]Department of Periodontology and Implant Dentistry, New York University College of Dentistry, New York, NY, United States

Background: Dysbiosis in oral microbial biofilms is considered a key risk factor for the onset of periodontitis. Cigarette smoking is a well-established risk factor for periodontitis, while the effect of using electronic cigarettes, especially its long-term impact on periodontal health, is not yet clearly understood.
Hypothesis: E-cigarette use may promote a dysbiotic salivary microbiome in periodontitis which is close to cigarette smokers and increase the infection risk.

Method: We conducted a longitudinal clinical study with two study visits, six months apart, to investigate the effect of e-cigarette use on the bacterial community structure in the saliva of 48 patients with severe periodontitis who are either cigarette smokers, e-cigarette users, or never smokers, using 16S rRNA gene sequencing method.

Results: Our data demonstrated that among those patients who were at the same periodontal disease stage, cigarette smokers and e-cigarette users shared more similarities in their salivary microbiota profile with altered species richness and community structures, which are distinct from the never smokers. E-cigarette smoking may have a similar potential as cigarette smoking at shifting the bacterial composition of saliva, leading to an increase in the relative abundance of periodontal disease-associated taxa such as *Filifactor* and *Treponema*.

Conclusion: Based on our findings, e-cigarette use promotes a smoking-alike salivary microbiome in periodontitis; therefore, we doubt the safety of using e-cigarettes as a less harmful alternative to combustible cigarettes with respect to oral health and dysbiosis.

10:55-11:15 AM
Oral Session III: Education

Rooms: 220 & 210

Oral Presentation 7

Helping students succeed on the Integrated National Board Dental Exam

Johanna Warshaw, Analia Veitz-Keenan, Lillian Moran, Eric Baker, Elena Cunningham

Background: Integrating foundational knowledge and clinical practice has long been a challenge in dental education. The need to implement changes in the curriculum is advisable for dental students to succeed on the Integrated National Board Dental Examination (INBDE) and to create a new generation of practitioners. Multiple opportunities to retrieve and review curricular content can help students work towards mastery and succeed on the INBDE.

Methods: A series of INBDE review courses is designed to support these requirements for the NYU College of Dentistry students, beginning in the Spring of their D1 year and continuing until the fall of their D4 year. These courses provide repeated opportunities, on a weekly basis in on-line quiz form, for students to retrieve knowledge from courses they have already taken. The quiz content, in both patient-box and stand-alone formats, and with associated feedback and review material, is contributed from faculty across College departments. It thus covers a comprehensive range of topics from throughout the curriculum in order to effectively allow students to review foundation knowledge, integrate it with clinical content, and prepare for the INBDE and clinical practice.

Results: Although the first cohort of students has yet to take the INBDE, student response to the INBDE review courses has been extremely favorable, with the majority of student survey respondents indicating that this helps them remember content from past courses. In addition, there has been a 100% pass rate for the course, with the average course grade an Honors Pass.

2:25-3:15 PM
Oral Session IV: Biomaterials

Rooms: 220 & 210

Oral Presentation 8

Deposition of Zinc Phosphate Solutions on Carious Dentin in Vitro

Andrea Shill[1], John Ricci PhD[1], Timothy Bromage PhD[1], Sasan Rabieh PhD[1], Marc Walters PhD[2]

[1]Department of Molecular Pathobiology; Division of Biomaterials, New York University College of Dentistry, NY
[2]Department of Chemistry, New York University, NY

Background: Dental caries affect about 2.4 billion people worldwide. A newer treatment to arrest carries with silver diamine fluoride (SDF) is shown to be safe and cost effective, but its practice is restricted due to a photochemical reaction and an aggregation of silver particles causing tooth discoloration. A potential alternative could be zinc phosphate solutions in aqueous and ethanol-based environments. Previous studies have shown the ability of zinc solutions to occlude tubules.

Hypothesis: We hypothesized that aqueous and ethanol-based zinc phosphate solutions will be able to form crystalized deposits to occlude tubules of carious molars.

Methods: Extracted permanent human molars with carious lesions were treated with either aqueous or ethanol-based zinc phosphate solutions. Solutions were painted onto the lesions using clinically standardized methods. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were used to map the location of zinc phosphate deposits in the dentin tubules. The speciation of the crystallizations were determined by X-ray powder diffraction (XRD).

Results: The zinc phosphate solutions were shown to form mineral deposits in dentin tubules. Images show the zinc occluded the surface of tubules and had robust depositions in the tubules. The XRD showed strong peaks indicating crystallinity.

Conclusion: These results show that there is zinc phosphate deposition and crystallization in dentin tubules. Since zinc has been shown to be antimicrobial, support remineralization, and does not discolor dentin, it could be potentially used to prevent and treat dental caries.

2:25-3:15 PM
Oral Session IV: Biomaterials

Rooms: 220 & 210

Oral Presentation 9

Nanoparticle-based, targeted delivery of PAR-2 antagonist in an in vivo inflammatory pain model

Gokul Sriman Thanigai Arasu[1,2], Rocco Latorre[2], Divya Bhansali[3], Kam W. Leong[3], Nigel Bunnett[1,2]

[1]Biomaterials Division, Department of Molecular Pathobiology[2], New York University, New York, NY
[3]Department of Biomedical Engineering, Columbia University, New York, NY

Background: We have previously shown that G-protein coupled receptors (GPCRs) such as protease-activated receptor-2 (PAR-2) are capable of carrying out pain signaling from endosomes. Currently, pain management largely relies on drugs targeting cell surface receptors which are ineffective in working against internalized receptors in different cellular compartments. Drugs and drug delivery modalities designed to target internalized pain receptors is a necessary to effectively combat chronic pain conditions.

Hypothesis: Pain management in mice model of inflammatory pain using poly(amidoamine) nanoparticles (PAMAM) loaded with PAR-2 antagonist is superior to using free PAR-2 antagonist

Materials and Methods: Loading of PAR-2 antagonists (AZ-3451) onto PAMAM-G3 was carried out by probe sonication followed by rotary evaporation and centrifugation. Release and loading of the drugs were confirmed using HPLC. PAR-2 antagonist loaded PAMAM NPs were evaluated using a PAR-2 induced inflammatory pain model and measuring upregulation of pro-inflammatory cytokines using qRT-PCR, immunofluorescence to assess PAR-2 internalization upon activation to the endosome and *in vivo* mechanical allodynia test in PAR-muGFP mice.

Results: We achieved significant reduction in inflammatory cytokine and pain in the animals treated with PAMAM loaded with PAR-2 antagonists compared to control animals.

Conclusion: Endosomal targeted antagonist alleviate inflammatory pain more effectively than antagonist targeting plasma membrane receptors.

Future directions: Established model of irritable bowel syndrome (IBD-like) in mice using trinitrobenzene sulphonic acid, dextran sodium sulphate to assess the efficacy of PAMAM-loaded PAR-2 antagonists to effectively inhibit PAR-2 endosomal signaling in chronic pain.

2:25-3:15 PM
Oral Session IV: Biomaterials

Rooms: 220 & 210

Oral Presentation 10

Slow-Release Nanoparticles for Chronic Pain Management via Intrathecal Delivery

Parker K. Lewis[1], Rachel Pollard[1], Rocco Latorre[2], Dane Jensen[2], Brian Schmidt[2], Nigel Bunnett[2], Nathalie Pinkerton[1]

[1]NYU Tandon School of Engineering, Department of Chemical and Biomolecular Engineering
[2]NYU College of Dentistry, Department of Molecular Pathobiology

Background: A reliable treatment for chronic pain has been elusive due to the rapid bodily clearance of traditional opioids. There exists a need for sustained intrathecal drug delivery to neuronal endosomes to target key pain-signaling pathways. Nanomedicine has shown promise in achieving prolonged drug release and endosomal uptake. Here, we develop a polymeric nanoparticle platform for sustained release of pain-signaling antagonists.

Hypothesis: Hydrophobic ion pairing (HIP) has demonstrated strong effects on release rates of small molecule drugs from polymeric nanoparticles. We hypothesized that employing hydrophobic counterions during nanoparticle assembly would increase the encapsulation efficiency of a calcitonin gene-like receptor (CLR) antagonist, and slow diffusion rate of this drug from the nanoparticle core.

Methods: The CLR antagonist was encapsulated into polymeric nanoparticles via Flash Nanoprecipitation (FNP). Synthesis parameters including counterion species, drug/counterion molar ratios, and solvent systems were varied to optimize nanoparticle drug loading (wt.%) and *in vitro* drug release rates.

Results: HIP of the CLR antagonist with pamoic acid resulted in nanoparticles (100 nm in diameter) with drug loadings of up to 6 wt.% and encapsulation efficiencies of up to 80%, with stability observed for over two weeks. These formulations demonstrated sustained drug release *in vitro* for over nine days.

Conclusions: The reproducible encapsulation of an antinociceptive drug demonstrates the ability for endosomal uptake by spinal neurons, preventing rapid clearance. In achieving high drug loading compositions and sustained drug release on the order of weeks, such formulations are optimal for the potent and prolonged inhibition of the CLR pain-signaling pathway.

3:35-4:25 PM
Oral Session V: Skeletal, Craniofacial & Developmental Biology

Rooms: 220 & 210

Oral Presentation 11

The core splicing factors EFTUD2, SNRPB and TXNL4A are essential for neural crest and craniofacial development

Byung-Yong Park, Melanie Tachi-Duprat^, Chibuike Ihewulezi, Arun Devotta and Jean-Pierre Saint-Jeannet

Background: Mandibulofacial dysostosis (MFD) is a human congenital disorder characterized by hypoplastic neural crest derived craniofacial bones often associated with outer and middle ear defects. There is growing evidence that mutations in components of the spliceosome are a major cause for MFD. Genetic variants affecting the function of several core splicing factors such as *SF3B4*, *EFTUD2*, *SNRPB* and *TXNL4A* are responsible for MFD in four related but distinct syndromes known as Nager and Rodriguez syndromes (NRS), mandibulofacial dysostosis with microcephaly (MFDM), cerebro-costo-mandibular syndrome (CCMS), and Burn-McKeown syndrome (BMKS), respectively. Animal models of NRS and MFDM indicate that MFD results from an early depletion of neural crest progenitors through a mechanism that involves apoptosis. Hypothesis: To determine whether MFD share a common root cause across multiple craniofacial spliceosomopathies we have analyzed the function of *Eftud2*, *Snrpb* and *Txnl4a* in *Xenopus* embryos using a morpholino-based knockdown approach.

Results: Our results show that interference with these factors cause altered gene expression in pre-migrating and migrating neural crest cells that correlates with increased apoptosis in the ectoderm. Later in development, these animals exhibited defects in neural crest derived craniofacial structures.

Conclusion: We propose that MFD associated with *EFTUD2*, *SNRPB* and *TXNL4A* haploinsufficiency has a common root cause, suggesting a universal mechanism underlying the etiology of craniofacial spliceosomopathies.

3:35-4:25 PM
Oral Session V: Skeletal, Craniofacial & Developmental Biology

Rooms: 220 & 210

Oral Presentation 12

Ca2+ clearance Mediated by Pumps (PMCAs) and Exchangers (NCKXs) in Enamel Cells

Guilherme H. Souza Bomfim[1] and Rodrigo S. Lacruz[1]

[1]Department of Molecular Pathobiology, New York University College of Dentistry, NY 10010, USA.

Background: Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) in enamel-ameloblast cells is finely controlled by the balance between Ca^{2+} influx (entry) and Ca^{2+} extrusion (clearance) mediated by a sophisticated Ca^{2+}-toolkit, including different plasma membrane pumps and exchangers. Ca^{2+}-ATPase (PMCA1-4) has been characterized a high Ca^{2+}-affinity and low Ca^{2+}-clearance capacity, while the K^+-dependent Na^+/Ca^{2+} exchangers (NCKX1-6) have low Ca^{2+}-affinity and high-Ca^{2+} capacity.

Hypothesis: Although PMCAs/NCKXs are expressed in secretory-SEC (matrix production) and maturation-MAT (mineral transport) stages, their specific role on regulation of Ca^{2+} homeostasis and mineralization remain unknown. Thus, this raised the issue of whether Ca^{2+} clearance thought PMCAs/NCKXs are involved in the maintenance of $[Ca^{2+}]_{cyt}$ or can mineralize the enamel-crystals.

Methods: We quantified gene expression by RT-qPCR and monitored $[Ca^{2+}]_{cyt}$ transients using ratiometric Ca^{2+}-probe and different approaches to activate/block the PMCAs/NCKXs in primary ameloblasts.

Results: PMCA1,3 mRNA levels were higher in secretory-SEC, while the NCKX4 expression was ~50-fold upregulated in maturation-MAT stage. Stimulating a small rise in $[Ca^{2+}]_{cyt}$, the ameloblasts showed relevant Ca^{2+}-clearance capacity thought PMCAs that was pharmacologically inhibited or potentiated, especially in secretory-SEC stage. By contrast, evoking a higher increase in $[Ca^{2+}]_{cyt}$ transients via Store-operated Ca^{2+} entry (SOCE), the NCKXs were responsible for the Ca^{2+}-clearance.

Conclusion: Collectively, our functional findings revealed that PMCA activity appear to more relevant in secretory-SEC stage, being involved in the basal $[Ca^{2+}]_{cyt}$ regulation. NCKXs cleared a higher amount of Ca^{2+} in maturation-MAT stage, suggesting a possible role in providing the enamel crystals with the required levels of Ca^{2+} during this stage hence contributing to enamel mineralization.

3:35-4:25 PM
Oral Session V: Skeletal, Craniofacial & Developmental Biology

Rooms: 220 & 210

Oral Presentation 13

MMP28 triggers EMT by regulating twist and cadherin-11 expression in neural crest cells

Nadege Gouignard, Eric Theveneau, Jean-Pierre Saint-Jeannet

Background: Epithelial-to-Mesenchymal Transition (EMT) is a complex process, which endows cells with migratory properties. It is a key process in embryogenesis and cancer progression. The EMT program appears to be highly context-dependent, which renders the task of agreeing on a unified definition across fields challenging. MMPs are secreted enzymes initially discovered for their extracellular matrix remodelling activity. Evidence showed that MMPs may influence EMT via their role in the extracellular space. However, MMPs are not considered as relevant markers or regulators of EMT but rather are studied for their role in later events such as cell migration. Given the widespread expression of MMPs by cells undergoing EMT, this calls for a re-assessment of their involvement in EMT, independently of their effects on extracellular matrix.

Methods: We used *Xenopus* neural crest (NC) cells to assess the putative role of MMP28 in EMT *in vitro* and *in vivo*.

Results: Our data show that, MMP28 is required for NC cells EMT. MMP28 knockdown causes downregulation of the EMT transcription factor twist and the cell adhesion molecule cadherin-11 leading to defects in NC cells adhesion and migration both *in vivo* and *in vitro*. The EMT program is partially or completely restored by expression of cadherin-11 and twist, respectively.

Conclusion: Our results demonstrate that MMP28 triggers the NC cells EMT program *in vivo* via the regulation of *twist* and *cadherin-11*. Our results challenge the paradigm on MMPs function during EMT and highlight the need for a reassessment of MMPs role during EMT in physiological and pathological context.

1:00-1:40 PM

Poster Session IA - Room A

P-1. Tackling PAR-mediated Cancer Pain with Nanocarriers

Divya Bhansali[1], Nguyen Tu[2], Nigel Bunnett[3], Brian Schmidt[2], Kam W. Leong[1]

[1]Department of Biomedical Engineering, Columbia University, New York, NY
[2]Bluestone Center for Clinical Research;
[3]Department of Molecular Pathobiology, New York University, New York, NY

Background: Oral cancer causes high pain levels in 70% of patients, affecting their day-to-day activity and quality of life. This pain worsens with disease progression and responds poorly to opioids. Recently, the protease-activated receptor-2 (PAR2) found in the endosome has been implicated in oral cancer pain.

Hypothesis: We explore the use of nanoparticles to deliver PAR2 receptor antagonists to the endosome as a therapeutic strategy to manage cancer pain.

Methods: PAMAM-G3 was functionalized with cholesterol to form an amphiphilic molecule that can self-assemble into a nanoparticulate drug delivery system. Nanoparticles were characterized by DLS and TEM and tagged with NHS-dye for fluorescent imaging. The uptake of fluorescent nanoparticles was studied using confocal microscopy. PAMAM-Chol nanoparticles were loaded with drugs via probe sonication and rotary evaporation. Loading and release were quantified using HPLC. Drug-loaded particles were evaluated with BRET and FRET analysis, calcium transients, and *in vivo* models of cancer pain.

Results: The nanoparticles were under 200 nm in size and over +30 mV in zeta potential in PBS, both with and without drug loading. Confocal imaging shows that the nanoparticles were uptaken and localized to endosomes within 1 hour. The AZ3451-loaded nanoparticles could effectively mediate PAR2 signaling *in vitro*. They could also mediate cancer pain in a dose-dependent manner in an *in vivo* model of cancer pain.

Conclusions: This study suggests the promise of an endosome-targeted nanomedicine for pain relief in oral cancer and other forms of PAR2-mediated pain.

1:00-1:40 PM

Poster Session IA - Room A

P-2. An Immunity Screen Assessing Covid-19 And Vaccination Induced Antibody Response At Point-Of-Care

Kritika Srinivasan Rajsri[a, b], Glennon W. Simmons[a], Michael P. McRae[a], Nicolaos J. Christodoulides[a], John T. McDevitt[a]*

[a]Department of Molecular Pathobiology, Division of Biomaterials, Bioengineering Institute, New York University College of Dentistry, New York, NY, USA
[b]Department of Pathology, Vilcek Institute, New York University School of Medicine, New York, NY, USA

Background: Coronavirus Disease – 2019 (COVID-19) has currently infected over 490 million people, leading to over 6 million deaths, worldwide. While COVID-19 vaccines are demonstrating robust seroconversion and disease prevention towards the fight to curtail this pandemic, accurate diagnosis of the causative agent – SARS-CoV-2 remains a key pillar to disease detection and monitoring. Antigen, antibody and molecular diagnostic testing have been used widely, but the virus continues to evolve in parallel, even as the society is pushing to achieve mass immunity - through vaccination, in addition to antibody response achieved post-infection.
Hypothesis: Individuals with immune compromise and suppression have been shown to have less than anticipated antibody response to COVID immunization, thus affecting their ability to mitigate disease. Understanding and quantitatively screening immune response is highly valuable not just in these individuals, but also for healthy populations. Sero-surveillance have insightful applications in diagnostics, relating to COVID vaccination and/or infection translated immune response. This information can help clinicians and scientists strategize vaccination dosage and therapeutics to mitigate disease risk and add essential value to vaccine perception and reception.

Methods: Utilizing an in-house lab-on a chip ecosystem, herein we present the proof-of-concept, optimization and validation of a POC strategy that quantitates the COVID-19 immunity screening.

Results: This platform covers the entire diagnostic timeline of the disease, seroconversion, and vaccination response spanning across single and multi-dosed vaccines - all in a single POC test. Initial test results demonstrate that this platform is rapid (~15 min), quantitative, and sensitive for SARS-CoV-2 specific IgG.

Conclusion: A quantitative serological assay, available at the point-of-care (POC), that measures anti-SARS-CoV-2 antibodies is beneficial to help screen the immune response in individuals' post vaccination and/or infection. Additionally, as vaccination continues to be implemented, rapid, accurate and quantitative POC COVID-19 testing remains critical for return to in-person operations.

1:00-1:40 PM

Poster Session IA - Room A

P-3. Highly Tunable Fluorescent Nanoparticle Library for Optimizing Neuronal and Gastrointestinal Drug Delivery

Rachel Pollard[1], Parker K. Lewis[1], Dane Jensen[2], Rocco Latorre[2], Shlok Joseph Paul[1], Brian Schmidt[2,3], Nigel Bunnett[2], Nathalie Pinkerton[1]

[1]NYU Tandon School of Engineering, Department of Chemical and Biomolecular Engineering
[2]NYU College of Dentistry, Department of Molecular Pathobiology
[3]NYU Langone Health, Oral Cancer Center

Background: Nanomedicine is a promising avenue for neuronal drug delivery, due to the favorable pharmacokinetics of encapsulated drugs, and potential for specific tissue targeting. However, nanoparticle-neuron interplay needs to be better understood in order to optimize these therapeutic systems. Here we present a tool to study these interactions.

Hypothesis: Previous studies indicated that size and surface properties play an important role in nanoparticle uptake by different cell populations. It was hypothesized that smaller, anionic particles would be endocytosed more efficiently than their counterparts.

Methods: A library of Quantum-Dot Nanoparticles (qNPs) was formulated by encapsulating CdSe-based quantum dots into polymeric nanoparticles using Flash Nanoprecipitation (FNP). Size was tuned by varying the amount of core polymer, and surface characteristics by altering the stabilizing polymer. Spatiotemporal qNP uptake was examined *in vitro* and *in vivo*.

Results: FNP was used to formulate a qNP library with tunability in size (50-250 nm diameter) and surface charge (-30 to +30mV). PEGylated qNPs showed size-dependent uptake rate into Schwann cells and colocalization with endosomes. In dorsal root ganglia, particles were visualized in both soma and neurites. In mouse colon, qNPs were taken up by intestinal epithelium and enteric nerves.

Conclusion: The qNP library is a novel tool that can be used for optimizing drug delivery systems and studying nano-bio interactions in any tissue system. It also demonstrates the versatility of FNP as a scalable single-step process for formulating nanocarriers. Preliminary studies confirm the ability of polymer nanoparticles to act as delivery systems to nerves via intrathecal and rectal delivery, and that tuning characteristics improves internalization.

1:00-1:40 PM

Poster Session IA - Room A

P-4. Physiochemical and bactericidal activity evaluation: Silver-augmented 3D-printed scaffolds - An in vitro study

Vasudev Vivekanand Nayak - Coelho/Witek Laboratory

Background: Injuries requiring resection of tissue followed by autogenous bone transfer may be prone to infection by *Staphylococcus aureus*, impeding recovery and increasing medical costs. For critical sized defects, the common approach to reconstruction is a tissue transfer procedure but is subject to limitations (e.g., donor site morbidity, cost, operating time).
Hypothesis: Utilizing beta-tricalcium phosphate (β-TCP) as bone grafting material augmented with silver (Ag), a custom graft may be 3D printed to overcome limitations and minimize potential infections.

Methods: Scaffolds were 3D printed and augmented with Ag by external attack on the surface by silver nitrate ($AgNO_3$) at varying concentrations (0.1, 1.0, 10% wt/wt of scaffold). The augmented scaffolds were evaluated utilizing X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and inductively coupled plasma mass spectroscopy (ICP-MS) to verify the presence of Ag and phosphate (PO_4) groups followed by electron microscopy, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) to gather evaluate physiochemical properties. Biocompatibility and bactericidal capacity of the scaffolds were tested using human osteoprogenitor cells and methicillin-sensitive *S. aureus* strain, respectively.

Results: Material characterization confirmed presence of Ag and PO_4 groups, whereas electron microscopy showed a decrease in Ca and an increase in Ag ions, decreasing Ca/P ratio with increasing surfactant concentrations. PrestoBlue assays yielded an increase in fluorescence cell counts among experimental groups with lower concentrations of Ag, whereas cytotoxicity was observed at higher concentrations. Antimicrobial evaluation showed reduced colony-forming units among all experimental groups when compared to 100% β-TCP.

Conclusion: β-TCP scaffolds augmented with Ag ions facilitate antibacterial effects while promoting osteoblast adhesion and proliferation.

1:00-1:40 PM

Poster Session IA - Room A

P-5. Effects of LED Curing Light on Silver Diamine Fluoride Penetration into Dentin

Cerezal G, Crystal YO, Rabieh S, Janal MN, Hmadeh N, Hu B, Bromage TG

New York University College of Dentistry, New York, NY

Background: Silver diamine fluoride's (SDF) recommended application time for caries arrest is 1-min. Empirical data suggests that using a curing light after application promotes immediate darkening of the tissue, indicative of caries arrest.
Aim: To evaluate the effects of LED light exposure on SDF penetration on primary teeth with deep carious lesions.

Hypothesis: Penetration of silver after LED light exposure following a 10 sec SDF application will be similar to a 1-min application.

Methods: Twenty-four teeth were allocated into 5 groups and treated as described within 5-minutes after extraction: (**1**) n=6, 1 drop of SDF applied for 1-min followed by 10 sec tap water rinse; **2**) n=6, 1 drop of SDF applied for 10 sec and exposed to LED light for 20 sec followed by 10 sec tap water rinse; (**3**) n=6, 1 drop of SDF applied for 10-sec followed by 1-min tap water rinse; (**4**) n=3, untreated; (**5**) n=3, untreated but exposed to LED light for 20 sec. Silver penetration was measured using scanning electron microscopy and energy-dispersive X-ray spectroscopy analysis.

Results: Mean ± SD penetration was: 86.4 ± 20.7% in Group 1, 94.3 ± 13.7% in Group 2, and 26.1 ± 14.7% in Group 3. Groups 1 and 2 were statistically similar and different from Group 3 ($p < .001$).

Conclusion: Use of LED light for 20-sec after 10 sec SDF application seems to facilitate the silver penetration, similar to a 1-min application. Clinical studies are needed to define the role of silver penetration in sustained caries arrest.

1:00-1:40 PM

Poster Session IA - Room A

P-6. Facilitating Faculty Collaboration and Integration of Didactic and Clinical Curriculum

Lillian Moran & Elena Cunningham

Background: A key initiative in the College's efforts towards integration of the curriculum is the INBDE prep courses. Integrating content from multiple areas, and spanning the four years of the curriculum, these courses give students repeated opportunities for knowledge retrieval and integration through weekly online quizzes (with unlimited submissions and targeted review material upon submission), end-of-semester exams, and mock INBDE exams; all of which consist of patient-cases and stand-alone questions. To facilitate creation of patient-cases and questions and collaboration among faculty, the authors conceived, designed, and developed a platform.

Methods: During the question creation process, the INBDE platform *requires* faculty to include meta-data and *encourages* the inclusion of review material. Each question is tagged with associated FKs and/or CCs, as defined by the *Joint Commission on National Dental Examinations* (2018). Additional required meta-data is captured (e.g. DDS course & semester, key systems area) allowing for the identification of the areas of the curriculum that are currently represented and areas of opportunity.

Results & Conclusion:

- over 600 questions (consisting of more than 100 patient-cases with approximately 470 associated questions, and over 150 stand-alone questions), and
- 35 DDS courses have contributed.

Faculty can utilize the platform to create questions, search existing questions, (allowing for a understanding of what is taught, and promoting a level of quality control and calibration in the curriculum), and add questions to existing patient boxes (facilitating communication and collaboration across courses, departments, and the four-years of the curriculum).

1:00-1:40 PM
Poster Session IB - Room B

P-7. SF3B2 knockdown disrupts neural crest cell development in Xenopus

Casey Griffin, Andrew Timberlake, Jean-Pierre Saint-Jeannet

Background: Craniofacial microsomia (CFM) is a syndrome of variable phenotype, most commonly including auricular malformations and underdevelopment of the mandible. The SF3B2 gene, which encodes a protein of the spliceosome, has been linked to patients with CFM; notably, patients with loss of function variants of SF3B2 show phenotypic heterogeneity consistent with the spectrum of CFM. The congenital anomalies seen in CFM suggest disturbance of cranial neural crest cell development, but the etiology of the disease has been unknown.

Hypothesis: Sf3b2 loss of function causes a depletion of neural crest progenitors and their derivatives.

Methods: We performed microinjection of morpholino antisense oligonucleotides to specifically interfere with Sf3b2 function in *Xenopus* embryos. We then analyzed the consequences on the expression of several neural crest genes at various stages of development by *in situ* hybridization, and the development of neural crest derived craniofacial structures at the tadpole stage

Results: Loss of Sf3b2 expression in the developing embryo results in a marked decrease in neural crest cell gene expression and subsequent reduction or loss of neural crest-derived craniofacial cartilages.

Conclusion: Targeted knockdown of Sf3b2 in *Xenopus* embryos indicates that cranial neural crest cell development is disrupted in the absence of Sf3b2 function, suggesting that neural crest depletion is a contributing factor to the pathology of CFM

1:00-1:40 PM
Poster Session IB - Room B

P-8. Cis-regulatory control of stage-specific notochord gene expression by Brachyury

Negrón-Piñeiro LJ[1,2], Kim D[2], and Di Gregorio A[2]

[1]Developmental Genetics Program, Vilcek Institute of Graduate Biomedical Sciences, NYU Grossman School of Medicine
[2] Department of Molecular Pathobiology, New York University College of Dentistry

Background: The notochord is an embryonic structure of mesodermal origin that functions as a signaling center and structural support for the surrounding embryonic tissues. Our lab uses an invertebrate chordate, *Ciona robusta,* to unravel the notochord gene regulatory network (GRN) and to characterize structure and function of notochord CRMs. The TF Brachyury is a main evolutionarily conserved regulator of notochord development. Despite being expressed specifically and continuously in the notochord throughout all the stages of its development, *Ciona Brachyury* (Ci-Bra) is somehow able to regulate the transcription of genes involved in sequential steps of notochord morphogenesis. Through the systematic characterization of 9 Ci-Bra-downstream notochord CRMs, our lab formulated a mechanistic hypothesis to explain how temporal information is encoded within Ci-Bra-downstream notochord CRMs.

Hypothesis: This hypothesis supports that Ci-Bra directly control early-onset genes through multiple cooperative and functional binding sites, middle-onset genes via a single functional binding site, while it indirectly controls late onset gene by other TFs acting as intermediaries, as they are devoid of functional Ci-Bra binding sites.

Methods: The main goal of this project is to test this hypothesis by identifying and characterizing Ci-Bra downstream notochord CRMs considering recently published sgRNA-Seq, ATAC-Seq chromatin profiles, and screenings performed on FACS-sorted *Ciona* notochord cells, integrating these results with the help of bioinformatic tools.

Results: The identification and characterization of newly identified Ci-Bra-downstream notochord CRMs revealed that 4 of them agree with our hypothesis in which the number of functional Ci-Bra binding sites matches their onset of expression, while 2 deviate from it. We are analyzing these regulatory sequences to elucidate the features that they contain and investigating whether other factors contribute to the temporal transcriptional regulation of Ci-Bra downstream genes.

1:00-1:40 PM
Poster Session IB - Room B

P-9. Role of SUCNR1 Signaling in Myeloid Lineage Homeostasis.

Prem Prakash, Xin Li

Department of Molecular Pathobiology, College of Dentistry, New York University.

Background and Hypothesis:
Succinate is an immuno-metabolite that accumulates in the cells under hypoxic and inflammatory condition. Once secreted out, it binds to the receptor named Succinate receptor 1 (SUCNR1), a GPCR protein on the cell surface and activates the downstream signaling cascade, and regulates the cellular inflammatory responses in diseases like periodontitis, cancer, and autoimmune diseases. Our previous work found that elevated succinate in serum is associated with the bone resorption and activates SUCNR1 on myeloid lineage cells to enhance osteoclastogenesis. However, its overall impact on the bone marrow cells and the underlying molecular mechanism is still not clear. Here, we aim to compare the impacts of succinate on the myeloid lineage cell differentiation and signaling of mouse bone marrow (BM) cells *in vivo* in the presence and absence of SUCNR1 using wild type (WT) and SUCNR1 global knockout (KO) mice.

Methods:
C57bl/6 (WT/KO) mice treated with PBS (Vehicle) and di-sodium succinate (4 mM/kg) daily for four weeks starting at the age of 8 weeks. Bar-coded single cells RNA samples were prepared and sequenced to analyze the transcriptomic profiling of various BM cell clusters.

Results:
Elevated succinate level observed at endpoint serum analysis in treated animals. Myeloid lineage clusters were identified. Succinate treatment did not alter the overall cell cycle of BM cells. Pathway enrichment analysis of BM myeloid cells support a distinctive response to succinate treatment in WT versus that in KO mice.

Conclusions:
This finding underscores the homeostatic role of SUCNR1 on myeloid lineages in contrast to no discernable impacts on other cell clusters.

1:00-1:40 PM
Poster Session IB - Room B

P-10. Abaloparatide at the same dose has the same effects on bone as PTH (1-34) in mice

Carole Le Henaff[1], Florante Ricarte[2], Brandon Finnie[1], Zhiming He[1], Joshua Johnson[1], Johanna Warshaw[1], Victoria Kolupaeva[2], Nicola C Partridge[1]

[1]Department of Molecular Pathobiology, New York University College of Dentistry, New York, NY, [2]Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY,

Background: Abaloparatide, a novel analog of parathyroid hormone-related protein (PTHrP1-34) became, in 2017, the second osteoanabolic therapy for the treatment of osteoporosis.

Hypothesis: This study aims to compare the effects of PTH(1-34), PTHrP(1-36), and abaloparatide on bone remodeling in male mice.

Methods: Intermittent daily subcutaneous injections of 80 µg/kg/day were administered to four-month-old C57Bl/6J male mice for six weeks. During treatment, mice were followed by DEXA-Piximus to assess changes in bone mineral density (BMD). At either four or eighteen hours after the final injection, femurs were harvested for µCT analyses and histomorphometry, sera were assayed for bone markers, and tibiae were separated into cortical, trabecular, and bone marrow fractions for gene expression analyses.

Results: Our results showed that, compared with PTH(1-34), abaloparatide resulted in a similar increase in BMD at all sites, while no changes were seen with PTHrP(1-36). With both PTH(1-34) and abaloparatide, µCT and histomorphometry analyses revealed similar increases in bone volume associated with an increased bone formation rate as shown by P1NP serum level and *in vivo* double labeling, and bone resorption as shown by CTX levels and osteoclast number. Gene expression analyses of trabecular and cortical bone showed that PTH(1-34) and abaloparatide led to different actions in osteoblast differentiation and activity and at different time points. Abaloparatide seems to generate a faster response on osteoblastic gene expression than PTH(1-34).

Conclusion: Taken together, abaloparatide at the same dose is as effective as PTH(1-34) as an osteoanabolic, with an increase in bone formation but also an increase in bone resorption in male mice.

1:00-1:40 PM
Poster Session IB - Room B

P-11. Adipocyte-specific deletion of FGF23 alleviates diet-induced obesity in mice

Min Young Park[1], Chia-Ling Tu[2], Wenhan Chang[2], Despina Sitara[1,3].

[1]Department of Molecular Pathobiology, New York University College of Dentistry, New York, USA
[2] San Francisco VA Medical Center, University of California San Francisco
[3]New York University School of Medicine, New York, USA

Background: Fibroblast growth factor 23 (FGF23) is a bone-derived hormone that regulates phosphate homeostasis. In addition to its central role in mineral balance, recent findings suggested that FGF23 is associated with chronic metabolic conditions including obesity. Elevated serum FGF23 levels are correlated with body fat mass, and consumption of high fat diet increases circulating FGF23 levels. However, the actions of FGF23 in adipocytes and its role in lipid metabolism have not been demonstrated.

Hypothesis: We hypothesize that adipocyte-specific ablation of FGF23 alleviates diet-induced obesity in mice.

Methods: We generated adipocyte-specific *Fgf23* null mice. Control (*Fgf23*$^{flox/flox}$) and knockout (KO, *Adiponectin*^{Cre}*Fgf23*$^{flox/flox}$) mice were fed either high-fat diet (HFD, 60 %kcal fat) or normal fat diet (10 %kcal fat) at 8 weeks of age for 24 weeks. Body weight measurements were taken every week and percentage of body fat was determined after 24 weeks of HFD. Serum, white adipose tissue, brown adipose tissue, and livers were collected upon sacrifice.

Results: After 24 weeks of HFD, body weight (BW) and body fat percentage, as well as fat weight/BW did not differ between control and KO male mice. However, female KO mice had lower body weight, percentage of body fat, and fat weight/BW compared to control mice. Also, decreased accumulation of triglyceride in the liver corresponded with downregulation of hepatic *PPARγ* and *CD36*, and reduced serum lipids in female KO mice.

Conclusion: Our results suggest that Fgf23 alleviates diet-induced obesity and regulates lipid metabolism in sex-specific manner.

1:00-1:40 PM
Poster Session IB - Room B

P-12. Long term exposure to methylene blue treatment in aged mice causes trabecular bone loss

Sher Bahadur Poudel[1], Gozde Yildirim[1], Manisha Dixit[1], Shoshana Yakar[1]*

[1]David B. Kriser Dental Center, Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY.

Methylene blue (MB) accumulates in mitochondria and acts as an electron acceptor/donor, carrying electrons from NADH and FADH2 to coenzyme Q (CoQ) and cytochrome C. Based on its chemical properties, we hypothesized that MB will enhance mitochondrial activity of the bone resident cells, osteocytes, and will protect from age-induced bone loss. Eighteen months old female C57BL/6J mice were given MB daily via drinking water for six months. We found that MB treatment did not affect body weight, or body composition. Skeletal parameters were assessed by micro-computed tomography (mCT) of the appendicular (femur) and axial (L5 vertebra) skeleton. Cortical bone parameters, taken at the femur mid-diaphysis, showed no significant difference between MB-treated and untreated groups. Surprisingly however, analyses of the trabecular bone compartment revealed decreases in bone volume, trabeculae thickness and bone mineral density (BMD) in MB treated mice. Comparable changes were seen in trabecular separation (Tb. Sp) between groups. I*n-vitro* cultures of osteoblasts derived from 24m old mice showed reduced cell viability and mitochondrial functions with MB treatment that were dose dependent. Our data indicate that MB treatment may have deleterious effects on regulation of bone mineralization and remodeling in aged mice.

1:40-2:20 PM
Poster Session IIA - Room A

P-13. PAR2 in chronic pain: From physiology to molecular characterization to structural study

Alan Hegron[1], Nigel Bunnett[1]

[1]Department of Molecular Pathobiology, New-York University, NY 10010

Background: Some proteases released during injury can activate the Protease-Activated Receptor 2 (PAR2) by cleaving its N-terminal part, leading to intracellular signaling pathways involved in hyperexcitability and nociception. Despite extensive studies on PAR2, no antagonist has been approved for human use.

Hypothesis: An extensive characterization of PAR2 and revealing its active structure will help develop new highly potent antagonists to treat chronic pain.

Methods: Our primary aim was to functionally characterize PAR2 in HEK293T and intestinal cells. We took advantage of mini-Gα proteins (mGαs) recognizing active conformations of GPCRs to characterize PAR2 at the cell surface and in endosomes using a bioluminescence resonance energy transfer (BRET) assay. Our second aim is to reveal the activated PAR2 structure using Cryo-EM. For that purpose, we expressed PAR2, extracted and purified it using styrene maleic acid lipid particles (SMALPs). We also purified Gαi proteins and ScFv16, a nanobody stabilizing the complex PAR2-Gαi.

Results: Results obtained by the functional characterization indicate that PAR2 activates Gαi and Gαq proteins and recruit Barrestin-2 from plasma membrane and endosomes. We also identified amino acids that play key role in ligand binding and PAR2 activation. For the cryo-EM part of the project, the purified PAR2, Gαi and ScFv16 can altogether form a functional complex.

Conclusion: The full characterization of PAR2 will allow to better understand its spatio-temporal activity and design new efficient strategies to target it. We now need to reveal the activated PAR2 structure by cryo-EM to allow the design of new drugs to target this receptor.

1:40-2:20 PM
Poster Session IIA - Room A

P-14. Pharmacological Inhibition of CGRP Receptor Reduces Cancer Nociception

Ammar Khan[1], Junhyeong Hwang[1], Nayab Khan[1], Jessy Dong[1], Sharanya Akula[1], Brandon Finnie[1], Jiho Lim[1], Nguyen Huu Tu[1], Brian L. Schmidt[1]

[1]Oral and Maxillofacial Surgery, Bluestone Center for Clinical Research, New York University College of Dentistry

Background: Cancer pain is debilitating, and current pain management options offer inadequate relief. Our previous findings have shown that calcitonin gene-related peptide (CGRP) plays a central role in periorbital nociception in mice. CGRP is a neuropeptide secreted from free nerve endings to tissues, including cancer tissue.

Hypothesis: We hypothesize that pharmacological inhibition of the CGRP receptor reduces cancer nociception in a pre-clinical cancer mouse model.

Methods: o generate the pre-clinical cancer mouse model, we inoculated 2 x 10^5 human oral squamous carcinoma cells (HSC-3, stock number JCRB0623, Japan) into the left hind paw of the NU/J *Foxn1^{nu}* mice (Catalogue number 002019, the Jackson Laboratories, Bar Harbor, ME). After the HSC-3 paw cancer mice developed cancer nociception, we treated the mice with a potent and selective CGRP receptor inhibitor (BIBN 4096, catalogue number 204697-65-4, TOCRIS, 1 mg.kg^{-1}, IP) or control. To measure cancer nociception in the cancer paw and to test the effect of CGRP receptor inhibitor on nociception, we used the paw von Frey assay.

Results: Our results showed that CGRP receptor inhibitor-treated mice had significantly reduced cancer nociception at 1-, 3- and 6-hours posttreatment. CGRP receptor inhibitor reduced cancer nociception by 56% (0.36 grams versus 0.20 grams) at 3 hours posttreatment, relieving the cancer nociception back to the mild to low nociceptive level.

Conclusion: This result suggests that pharmacological inhibition of the CGRP receptor adequately reduces cancer nociception. Our findings establish the foundation for the development of new treatment approaches that target the CGRP receptor in oral cancer pain.

1:40-2:20 PM
Poster Session IIA - Room A

P-15. SOCE regulates MMP-1 expression modulating the transformation of dysplasia to oral cancer.

Ga-Yeon Son[1], Nguyen H. Tu[2], Youssef Idaghdour[3], Brian L. Schmidt[1, 2], and Rodrigo S. Lacruz[1]

[1]Department of Molecular Pathobiology, New York University College of Dentistry, New York, NY, 10010, USA. [2]Bluestone Center for Clinical Research, New York University College of Dentistry, New York, NY, 10010, USA. [3]Program in Biology, Division of Science and Mathematics, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates.

Background: Store-operated Ca^{2+} entry (SOCE) mediated by the ORAI channels is critical for cell function such as proliferation, cell death, and gene regulation through the activation of the nuclear factor of activated T-cells (NFAT). ORAI channels are activated by endoplasmic reticulum (ER)-resident stromal interaction molecules (STIM) proteins following ER-Ca^{2+} depletion. In most non-excitable cells, Ca^{2+} influx is strongly regulated by SOCE. The expression levels of ORAI (1-3) and/or STIM (1-2) isoforms is up-regulated in various cancer types. However, the associations between SOCE and oral cancer progression are poorly understood.

Aim: We evaluated SOCE in several human oral cancer cells and controls, including dysplastic cells, to address the role of SOCE in oral cancer progression focusing on the expression of *MMP-1,* a marker of the malignant transformation of oral dysplasia to oral cancer.

Materials and methods: Human oral cancer cells (HSC-3, SCC-9), dysplastic oral keratinocyte (DOK) and normal keratinocytes (HaCaT) were used to determine the mRNA levels of *ORAI* and *STIM* isoforms, as well as, *MMP-1*, using qPCR. We measured SOCE using cyclopiazonic acid (CPA)/thapsigargin, and monitored changes in cytosolic Ca^{2+} concentration after Ca^{2+} re-addition. SOCE was measured in the presence/absence of the ORAI blocker synta66, in sh*ORAI1* and CRISPR/Cas9 *ORAI1* HSC-3 cells. We also quantitated the effects of loss of ORAI1 function in cell proliferation. To address global changes in genes and pathways modulated by SOCE, we performed an RNA-Seq of HSC-3 cells after SOCE stimulation in the presence/absence of synta66 and the NFAT blocker cyclosporine-A (CsA).

Results: All cells expressed the mRNA levels of *ORAI1-3* and *STIM1-2* and showed functional SOCE that was inhibited by synta66 and *Orai1* KD. *ORAI1* down-regulation decreased cell proliferation without affecting cell death. Stimulating SOCE with CPA increased *MMP-1* expression that was down-regulated by pre-treatment with synta66 or CsA. Over-expression of ORAI1 and STIM1 in DOK cells increased *MMP-1* expression with and without stimulating SOCE. RNA-Seq data showed an overall increase in the expression of *MMP* genes (*MMP*-1, 3, 9, 10, 12, 13, 14, 15) in SOCE stimulated HSC-3 cells that was down-regulated by synta66 or CsA treatment.

Conclusions: Our findings support the role of SOCE as important for Ca^{2+} influx in human oral cancer cells and their proliferation. Moreover, we found that *MMP-1* expression and other isoforms used as genetic markers in oral cancer progression are modulated by SOCE, and are downstream from NFAT activation. These data suggest that SOCE is an important mediator in the malignant transformation of oral dysplasia to oral cancer.

1:40-2:20 PM
Poster Session IIA – Room A

P-16. ATP synthase is essential for the high-conductance mitochondrial permeability transition but not for stress-induced mitochondrial depolarization
Maria A. Neginskaya, Sally E. Morris, Evgany Pavlov

Mitochondrial permeability transition (mPT) is caused by the opening of the Cyclosporin A (CSA) dependent calcium-induced large pore, known as the Permeability Transition Pore (PTP). PTP activation is believed to be a central event in cell death during the stroke and heart attack. However, the molecular details of PTP opening remain incompletely understood. PTP opening makes the mitochondrial inner membrane permeable to the molecules up to 1.5 kDa in size. Solute equilibration due to the PTP opening makes mitochondria optically transparent. Here, we utilized holographic microscopy to monitor mitochondrial optical density (OD) changes caused by PTP induction and, thus, to determine the molecular mechanism of mPT inside the living cells. PTP activation was detected as the decrease in mitochondrial OD. Mitochondrial membrane potential was monitored in parallel, using the fluorescent probe TMRM. In intact HAP 1 cells, we found that calcium stress caused CSA-sensitive depolarization of mitochondria. Unexpectedly, high-conductance PTP did not occur until after nearly complete mitochondrial depolarization. In cells lacking c and δ subunits of the ATP synthase, we observed calcium-induced and CSA-sensitive depolarization but not high-conductance PTP. We **conclude** that in living cells, high-conductance PTP is not the cause of calcium-induced membrane depolarization. Further, we provide direct evidence that ATP synthase is essential for high-conductance PTP, but not for calcium-induced CSA-sensitive membrane depolarization. We propose that PTP activation occurs as a two-phase process, where the initial depolarization is followed by a large pore opening that results in membrane permeabilization.

1:40-2:20 PM
Poster Session IIA – Room A

P-17. ELB00824, a novel PPAR gamma agonist, prevents oxaliplatin-induced pain and reduces spinal oxidative stress.

Morgan Zhang[1,2,] Min Hu[3], Marena A. Montera[5], Karin N. Westlund[5], Ian Adams[5], Sascha R. A. Alles[5], Yi Ye[1,2]

[1]Bluestone Center for Clinical Research, New York University College of Dentistry, 421 First Avenue, 233W, New York, NY, 10010, USA.
[2]Department of Molecular Pathobiology, New York University College of Dentistry, 345 E. 24th street, New York, NY 10010, USA
[3]Shanghai Elixiria Biotech Co. Ltd, 578 Yingkou Road, Yangpu District, Shanghai 200433, China
[5]Department of Anesthesiology & Critical Care Medicine, MSC10 6000, 2211 Lomas Blvd. NE, University of New Mexico Health Sciences Center, Albuquerque, NM 87131, USA

Background: Chemotherapy-induced neuropathic pain (CINP) is a debilitating and difficult-to-treat side effect of chemotherapeutic drugs (e.g., oxaliplatin). CINP is marked with oxidative stress and neuronal hypersensitivities. The peroxisome proliferator-activated receptor gamma (PPARγ) is a transcription factor that regulates genes involved in oxidative stress and inflammation.

Hypothesis: PPARγ agonists are protective against CIPN by reducing oxidative stress and inhibiting neuronal hypersensitivities.

Methods: Acute or chronic CIPN was introduced by short or long-term treatment of oxaliplatin in BALB/c mice. A novel BBB (blood-brain barrier) penetrable PPARγ agonist ELB00824, a BBB non-penetrable PPARγ agonist pioglitazone, or vehicle was IP injected 5 min before each oxaliplatin treatment. Cold allodynia, mechanical allodynia, motor coordination, sedation and addiction were measured with dry ice, von Frey filaments, beam-walking tests, and conditioned place preference, respectively. Oxidative stress was accessed by measuring spinal levels of byproducts of protein oxidation (carbonyl and 3-Nitrotyrosine), and lipid peroxidation [Thiobarbituric acid reactive substances (TBARS)]. Neuronal hypersensitivities were measured using whole-cell current clamp recordings in isolated dorsal root ganglion (DRG) neurons.

Results: In both acute and chronic CIPN models, ELB00824, but not pioglitazone, reduced oxaliplatin-induced cold and mechanical allodynia and spinal oxidative stress. ELB0824 suppressed oxaliplatin-induced firing in IB4- neurons. ELB00824 did not cause motor discoordination or sedation/addiction or reduce the antineoplastic activity of oxaliplatin (measured with an MTS-based cell proliferation assay) in human colon (HCT116) or oral (HSC-3) cancer cell lines.

Conclusion: ELB00824 prevents oxaliplatin-induced pain, likely via inhibiting both DRG neuronal hypersensitivities and spinal oxidative stress.

1:40-2:20 PM
Poster Session IIB – Room B

P-18. Modulation of the spatiotemporal dynamics of NGF-mediated signaling

Chloe J. Peach, Nigel W. Bunnett

Background. Nerve growth factor (NGF) primarily signals *via* tropomyosin receptor kinase A (TrkA), a receptor tyrosine kinase (RTK) highly expressed in nociceptive primary afferent neurons. NGF/TrkA signals from endosomes in sympathetic neuronal development, however few studies have linked NGF/TrkA endosomal signaling to its ability to sensitize nociceptors in pain. An increasing number of growth factors co-interact with neuropilin-1 (NRP1), a transmembrane protein that modulates other RTKs. It is also unknown whether neighboring proteins, such as NRP1, modulates TrkA signaling.

Hypothesis. NRP1 modulates NGF/TrkA signaling in pain.

Methods. For *in vitro* studies, cells were transfected with TrkA in the absence or presence of NRP1. ERK signaling was monitored using a downstream transcriptional luciferase reporter or a real-time localized FRET biosensor. Receptor localization was also monitored in live cells using tagged TrkA in the presence of NRP1. To investigate NGF-induced signaling *in vivo*, wild-type mice were monitored for mechanical allodynia and thermal hyperalgesia.

Results. NGF induced a concentration-dependent increase in ERK transcription and real-time signaling in HEK293 cells. NRP1 co-expression increased the potency of NGF to induce ERK-mediated gene transcription, as well as real-time ERK signaling at low NGF concentrations. Confocal imaging demonstrated TrkA and NRP1 were highly colocalized. In a behavioral pain assay, peripheral co-administration of NGF with a small molecule inhibitor of NRP1 (EG00229, 30 µM/10 µl, intraplantar) reversed NGF-induced sensitization for up to 2 hours.

Conclusion. There is a novel link between NGF-induced TrkA signaling and NRP1, whereby NRP1 enhances ERK signaling and nociception through an unknown mechanism.

1:40-2:20 PM
Poster Session IIB – Room B

P-19. Identification of a novel neuropilin 1 inhibitor that blocks CRMP2 phosphorylation and reverses mechanical allodynia and thermal hyperalgesia in a rodent model of neuropathic pain

Paz Duran[1,2], Harrison Stratton[3], Lisa Boinon[3], Laurent Martin[4], Kimberly Gomez[1,2], Yuan Zhou[3], Dongzhi Ran[3], Shizhen Luo[3], Samantha Perez-Miller[1,2], Mohab Ibrahim[4], Amol Patwardhan[4], Aubin Moutal[5], Rajesh Khanna[1,2,5]

[1]Department of Molecular Pathobiology, College of Dentistry, New York University, New York, New York, United States of America
[2]NYU Pain Research Center, 433 First Avenue, New York, NY 10010, United States of America
[3]Department of Pharmacology, College of Medicine, The University of Arizona; Tucson, Arizona, 85724 United States of America.
[3]Department of Anesthesiology, College of Medicine, The University of Arizona; Tucson, Arizona, 85724 United States of America.
[5]Saint Louis University - School of Medicine, Department of Pharmacology and Physiology, 1402 S. Grand Blvd., Schwitalla Hall, Room 432, Saint Louis, MO 63104

Background: Chronic pain is a major societal burden with few therapies that are both efficacious and safe. We previously showed that blockade of the interaction between the cell surface receptor Neuropilin 1 (Nrp1) and vascular endothelial growth factor A (VEGF-A), produces antinociception in a rodent model of neuropathic pain.

Hypothesis: Here, we focused on developing a class of Nrp1 inhibiting compounds with the potential to alleviate pain.

Methods: Using an in silico docking approach, we screened a library of ~480K small molecules and identified nine chemical series with lead- or drug-like physico-chemical properties. From these hits, compound NRP1-4 was selected for further evaluation using electrophysiology, synaptic fractionation and both spared nerve injury and spinal nerve ligation rodent models of neuropathic pain.

Results: Synaptic fractionation of rat spinal cord tissue revealed that treatment with NRP1-4 significantly attenuated the phosphorylation of the collapsin response mediator protein 2 (CRMP2) at Ser522 without affecting total CRMP2 expression levels. Furthermore, NRP1-4 did not affect the synaptic membrane localization of the pain relevant ion channels NaV1.7 or CaV2.2, both of which are regulated by CRMP2. Whole cell sodium currents and N-type calcium currents in cultured primary sensory neurons were potentiated by treatment with 1nM VEGF-A, but blocked by co-treatment with 12.5 µM of NRP1-4. Ex vivo evaluation of synaptic activity showed that NRP1-4 reduced VEGF-A-mediated increases in both the frequency and amplitude of spontaneous excitatory post synaptic currents (sEPSCs). Administration of NRP1-4 significantly attenuated both mechanical allodynia and thermal hyperalgesia in rodent models of neuropathic pain.

Conclusion: Together, our findings show that NRP1-4 is a first-in-class compound targeting the VEGF-A/NRP1 interaction and its action mechanism involve signaling upstream of CRMP2 to control ion channel activities and curb chronic pain syndromes.

Poster Session IIB – Room B

P-20. mPTP induction by polyP is chain length-dependent

Yaw A. Akosah, Vedangi Hambardikar, Maria E. Solesio, Evgeny V. Pavlov

Mitochondrial permeability transition pore (mPTP) refers to a large channel of the inner mitochondrial membrane that activates during oxidative stress and/or elevated concentrations of mitochondrial calcium. mPTP opening leads to rapid loss of the mitochondrial membrane potential and the consequent loss of adenosine triphosphate. Recently, the possible role of inorganic polyphosphate (polyP) in the activation of mPTP has been proposed. PolyP activation of mPTP might occur through regulation of bioenergetics, Ca^{2+} buffering, and/or direct participation in mPTP channel formation. In this study, we investigated the potential role of PolyP in the induction of mPTP in SH-SY5Y cells. Using a recently developed holographic imaging assay, we tracked the induction of mPTP in wild-type (wt) cells, and mutants overexpressing exopolyphosphatase (mitoPPX) or endopolyphosphatase (mitoPPN). Following the treatment of cells with ferutinin (a calcium ionophore), the influx of Ca^{2+} triggered the depolarization and permeabilization of the mitochondrial membrane in wt and mitoPPX cells. Although treatment of mitoPPN cells (in which long-chains of polyP have been depleted) led to mitochondrial depolarization, the induction of mPTP was not observed. These preliminary data suggest that long-chain polyP is required for the induction of mPTP. We **hypothesize** that polyP involvement in mPTP is likely linked to its direct formation of the mPTP channel rather than to its regulation of the bioenergetics or Ca^{2+} buffering capacity. This hypothesis will be further tested in future experiments.

1:40-2:20 PM
Poster Session IIB – Room B

P-21. Pentacyclic triterpenoids inhibit N- and T-type voltage-gated calcium channels to attenuate nerve-injury associated neuropathic pain

Aida Calderon-Rivera[a], Kimberly Gomez[a], Santiago Loya-Lopez[a], Harrison Stratton[a], Cheng Tang[a], Paz Duran[a], Kyleigh Masterson[b], Omar Alsbiei[b], Aubin Moutal[b], Samantha Perez-Miller[a], Leslie Gunatilaka[c], Rajesh Khanna[a]

[a]Department of Molecular Pathobiology, NYU Pain Research Center, New York University, New York, NY, United States.
[b]Department of Pharmacology, College of Medicine, The University of Arizona, Tucson, AZ, United States.
[c]Natural Products Center, School of Natural Resources and the Environment, College of Agriculture and Life Sciences, The University of Arizona, Tucson, AZ, United States.

Background: Natural products usage has increased in the last three decades almost 80% of people worldwide relying on them for some part of primary healthcare. Over the last decade, our laboratories have isolated and characterized natural products with analgesic properties from arid land plants or their associated fungi.

Hypothesis: A pentacyclic triterpenoid derivative of Betulinic acid (BA) isolated from the desert lavender *Hyptis emoryi* modulates Voltage-gated calcium (Ca^{2+}) channels activity and attenuates neuropathic pain.

Methods: We previously reported that BA attenuates paclitaxel-, HIV-, and nerve injury-associated peripheral sensory neuropathy via block of N- and T-type Ca^{2+} channels. Using structure activity relationship (SAR) data, docking studies and virtual screening of BA analogs mined from the ZINC20 database, we designed second-generation BA analogs (BA-II analogs) with unique intellectual property and improved predicted PK properties. Here, we tested BA-II analogs for their in vitro mechanism of action on voltage-gated calcium channels in rat dorsal root ganglia (DRG) neurons using ratiometric calcium imaging and electrophysiology; and in vivo in the spared nerve injury (SNI) model of neuropathic pain.

Results: Screening of the 30 BA-derivatives identified one BA-II analog (NPC 1-11) with significant inhibition of Ca^{2+} influx in DRGs. NPC 1-11 significantly downregulated total Ca^{2+} currents on DRGs without affecting voltage-dependence activation and inactivation curves. Inhibition by NPC-1-11 was limited to (CaV2.2) N- and T- type Ca^{2+} currents. Intrathecal delivery of NPC 1-11 (2µg/5µl) reversed mechanical allodynia induced by SNI.

Conclusion: Our in vitro and in vivo results demonstrate that inhibition of Ca^{2+} channels by the natural product BA-derivative alleviates neuropathic pain. Because of the wide-safety profile of pentacyclic triterpenoids, the mechanistic insights gleaned from these studies has the potential to lead to fast-tracking development of novel, non-addictive drugs for treatment of chronic neuropathic pain in humans.

1:40-2:20 PM
Poster Session IIB - Room B

P-22. Serotonin Of The Intestinal Mucosa Alleviates Anxiety And Depression Independently Of Gastrointestinal Function And The Ens

Lin Y. Hung[1] Andrew Del Colle[1], Nuno Alves[2], Zixing Huang[1], Sarah Najjar[1], Narek Israelyan[2], Ruxandra Tonea[4], Kimberly Sigety[1], Christian Bury[1,5], Melissa Medina[5], Marguerite Bernard[5], Ray Rahim[1], Daniel Juarez[6], Roey Ringel[6], Michael Gershon[7], Mark Ansorge[2], Kara Gross Margolis[1,5]

[1]Department of Pediatrics, Morgan Stanley Children's Hospital, Columbia University Irving Medical Center, [2]New York State Psychiatric Institute, Columbia University, [3]Vagelos College of Physicians and Surgeons, [4]Fu Foundational School of Engineering & Applied Science, Columbia University, [5]Institute of Human Nutrition, Columbia University Irving Medical Center, [6]Columbia College, Columbia University, [7]Department of Pathology and Cell Biology, Columbia University Irving Medical Center

Depression and anxiety are common debilitating mood disorders that affect up to 8% of individuals in the U.S. Selective serotonin reuptake inhibitors (SSRIs), which increase serotonin bioavailability, are among the first line of drugs used to treat mood disorders. SSRIs inhibit the plasmalemmal serotonin transporter (SERT) and thus impede the transmembrane transport of serotonin into cells. Because uptake is the primary mechanism of serotonin inactivation, SSRIs enhance and prolong serotonergic transmission. Despite the therapeutic benefits of SSRIs for mood disorders, there are limitations: (1) systemic absorption, (2) off-target adverse effects, (3) inadequate efficacy (<50% remission after SSRI exposure) and (4) increased risk of childhood mood and functional gastrointestinal (GI) disorders due to *in-utero* SSRI exposure. It is not known whether the manyfold actions of SSRIs are due to effects manifested in brain or gut, where most of the body's serotonin is found (>95%). Within the bowel, SERT terminates actions of mucosal and enteric nervous system (ENS) serotonin. Each of these serotonin stores affect GI motility, ENS development, and inflammation and the function of each may be opposite to the other. To date, no prior study has distinguished effects of mucosal from ENS serotonin on brain and mood. To test the idea that selective elimination of mucosal or ENS SERT affects mood and GI function differentially, we created transgenic mice in which SERT is ablated in targeted layers of the bowel wall. In *Villin^{Cre}::SERT$^{fl/fl}$* mice, SERT is eliminated only in the mucosa, while in *Wnt1^{Cre}::SERT$^{fl/fl}$* mice, SERT is eliminated only in the ENS. Both *Villin^{Cre}::SERT$^{fl/fl}$* and *Wnt1^{Cre}::SERT$^{fl/fl}$* mice and their wildtype littermates were examined for mood disorders (anxiety and depression), *in vivo* GI motility, colonic migrating motor complex (CMMC) frequency, and ENS phenotypic expression (immunocytochemistry). Mice lacking SERT selectively in the ENS displayed hyperplasia of neurons and serotonergic varicosities. GI dysmotility was also evident in these *Wnt1^{Cre}::SERT$^{fl/fl}$* mice; total GI transit accelerated, CMMC frequency increased over control, while time to expel beads from the colon was paradoxically slow. Behavioral tests revealed that anxiety and depression were prominent in *Wnt1^{Cre}::SERT$^{fl/fl}$* mice. In contrast to *Wnt1^{Cre}::SERT$^{fl/fl}$* mice, Villin^{Cre}::SERT$^{fl/f}$ mice displayed an anti-anxiety and non-depressive phenotype; nevertheless, *in-vivo* GI motility, CMMC frequency and enteric phenotypic expression were not significantly different from controls. Our observations reveal that selective deletion of SERT in the GI mucosa, with preservation of SERT in the CNS and ENS, is sufficient to ameliorate anxiety- and depression-like phenotypes without causing deleterious effects on GI function and ENS development.

1:40-2:20 PM
Poster Session IIB - Room B

P-23. Early life adversity alters enteric nervous system development, gut motility, and visceral sensitivity

Sarah Najjar, Kara Gross Margolis

Background: Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal (GI) disorder that affects individuals worldwide. Predisposition to IBS development has been linked to early-life adversity. A well-established preclinical model of early-life adversity is the maternal separation (MS) model, which results in symptoms mimicking IBS including intestinal hyperpermeability and hypersensitivity, microbiota dysbiosis, and psychiatric comorbidities. These gut and behavioural changes are consistent with the hypothesis that there are brain-gut communication defects in patients with IBS and we sought to investigate potential changes in enteric nervous system (ENS) development and gut motility function.

Hypothesis: MS results in gut motility defects caused by developmental abnormalities in the ENS.

Methods: C57BL/6 mice were separated from their mothers for 3 h per day from postnatal day 2 through 12. At 8 weeks of age, all mice underwent *in vivo* testing for total-gastrointestinal-transit (TGIT) time colonic motility, gastric-emptying (GE) and small intestinal transit (SIT), and visceral sensitivity to colorectal distension. The ENS was analysed by immunohistochemistry.

Results: MS resulted in faster GI transit in female mice and slower transit in male mice. Both sexes exposed to MS exhibited visceral hypersensitivity. MS mice displayed ENS hyperplasia and differences in cholinergic and serotonergic neurons, suggesting that developmental changes could contribute to the gut motility defects.

Conclusions: Early-life adversity alters ENS development and gut motility. Therapies for the GI dysfunction that result from early-life adversity in may be targeted specifically to the ENS.