THE NINETEENTH ANNUAL
BASIC SCIENCES RESEARCH SYMPOSIUM
Jointly with
THE FIFTH ANNUAL
INLAND EMPIRE STEM CELL CONSORTIUM
SYMPOSIUM

Tuesday, November 8, 2016

CENTENNIAL COMPLEX

LOMA LINDA
UNIVERSITY

A science symposium organized by the

BASIC SCIENCE DEPARTMENT
SCHOOL OF MEDICINE
LOMA LINDA CA 92354

POSTER ROSTER BY LOCATION

Poster #	Last Name	First Name
1	Ximinies	Alexia
2	Ball	Kate
3	Williams	Paul
4	Burchell	Sherrefa
5	Watson	Billy
6	Burnham	Leanne
7	Walemba	Elvin
8	Chen	William
9	Stoll	Shaunrick
10	Desai	Arti
11	Sanchez	Nicholas
12	Esebanmen	Grace
13	Rowland	Leah
14	Kabagwira	Janviere
15	Hile	Thomas
	Mutiso	Rose
16	King	Joshua
17	Mukosera	George
18	Li	Xiaolan
19	Lindsey	Richard
20	Licero Campbell	Jenniffer
21	Lee	Jeong Bin
22	Mcmullen	James
23	Gonda	Amber
24	Moyron	Ron
25	Esiaba	Ijeoma
26	Sackett	Jonathan
27	Cooper	Jamey
28	Vega-Torres	Julio D
29	Campbell	Petreena
30	Whang	Sonia
31	Cajigas-Du Ross	Christina
32	Winter	Catherine
33	Baio	Jonathan
34	Wang	Hanmin
35	Doycheva	Desislava
36	Ahmed	Abu
37	Ahmed	Abu
38	Baghchechi	Mohsen
39	Angeles	Danilyn
40	Forde	Dorothy

Poster #	Last Name	First Name
41	Coats	Jacquelilne
42	Haddad	Elizabeth
43	Gamboa	Yaritxa
44	Mao	Xiao Wen
45	Jullienne	Amandine
46	Milford	Terry-Ann
47	Perry	Christopher
48	Sinclair	Ryan
49	Stanbouly	Seta
50	Tang	Xiaolei
51	Vidales	Veriah
53	Vlkolinsky	Roman
54	Wasnik	Samiksha
55	Pecaut	Michael
56	Kirsch	Wolff
57	Nishiyama	Nina
58	Wilson	Aruni
59	Chen	Wanqiu
60	Xu	David Yi
61	Gomez	Gustavo
62	Maldonado	Maricela
63	Hong	Yiling
64	Weng	Nikki
65	Maldonado	Maricela
66	Taguchi	Takashi
67	Dana	Rutherford
68	Zhang	Naiyin
69	Loza	Antonio
70	Ubina	Teresa
71	Mccarthy	Pierce
72	Tian	Qiaomu
73	Sandhu	Nabjot
74	Shelar	Patrick
75	Soria Jr.	Pedro
76	Salin	Joshua
77	Toomey	Channing
78	Mambo	Nathaniel
79	Zahedi	Atena

STUDENT POSTER LOCATIONS BY SESSION

SESSION 1

LLU Basic Sciences

Poster #	Last Name	First Name
1	Ximinies	Alexia
3	Williams	Paul
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9	Stoll	Shaunrick
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Inland Empire Stem Cell Consortium

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69	Loza	Antonio
71	Mccarthy	Pierce
73	Sandhu	Nabjot
75	Soria Jr.	Pedro
77	Toomey	Channing
79	Zahedi	Atena

SESSION 2

LLU Basic Sciences

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74	Shelar	Patrick
76	Salin	Joshua
78	Mambo	Nathaniel

1

Student

Studies on the role of the novel PG0686 protein in response to Environmental Stress in *Porphyromonas gingivalis* W83

Alexia D. Ximinies, Yuetan Dou, Wilson Aruni, Lawrence Sandberg, Francis Roy and Hansel M. Fletcher

Loma Linda University

Porphyromonas gingivalis is an important periodontopathogen. Its survival in the inflammatory environment of the periodontal pocket requires an ability to overcome oxidative stress (OS) caused by reactive oxygen species (ROS). Transcriptome analysis in P. gingivalis demonstrated that OS modulates several functional classes of genes according to severity and duration of exposure. There was a 4.0 up-regulation of the hypothetical protein, PG0686, in the presence of 0.25 mM hydrogen peroxide for 15 minutes. In other studies PG0686 was modulated by 6% oxygen (O2) and nitric oxide (NO). The purpose of this investigation is to evaluate the role of hypothetical protein, PG0686 in OS resistance in P. gingivalis. Using PCR fusion, upstream and downstream flanking regions of the PG0686 gene were fused with the ermF antibiotic cassette, creating a PG0686-defective mutant, designated FLL361. Real-time-PCR confirmed upregulation of PG0686 by 8.16, 3.85, and 1.99 fold when P. gingivalis was exposed to 0.25 mM H2O2, 21% O2 (air), and 24mM NO, respectively. Similar to wild-type W83, FLL361 was black-pigmented and beta hemolytic on Brucella blood agar. Lysine-specific gingipain activity of FLL361 was reduced by 25% and 37% in log and stationary phases, respectively; while arginine-specific gingipain activity remained unchanged compared with W83. FLL361 was more sensitive to H2O2 and O2 compared to W83. In silico analysis of the PG0686 protein identified 6 domains, including a hemerythrin domain, DUF-1858 (domain of unknown function), and a sensory box domain. The ORF of the PG0686 gene was cloned into the pEXP5-NT vector, and the 60 kDa histidine-tagged recombinant-PG0686 protein was overexpressed and purified. A mechanism(s) for the specific role of the PG0686 protein in OS resistance is under investigation. Our preliminary data indicate that PG0686 may be important in OS resistance in Porphyromonas gingivalis W83, and may function in the response to multiple types of environmental stress.

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Student

Curcumin Promotes Cell Death through Caspase-Independent Apoptotic and Necroptotic Factors in PANC-1 Cells

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Pancreatic cancer is the third leading cause of cancer-related deaths in the US and the American Cancer Society predicts it will claim over 41,000 lives by the end of 2016. Chemotherapy resistance is an important issue in many pancreatic cancer patients leading to high mortality rates. PANC-1 pancreatic cancer cells have demonstrated resistance to known chemotherapies and are a good model for chemotherapy-resistant cancer. Chemotherapy resistance can be facilitated by Inhibitors of Apoptosis (IAPs) such as Survivin, XIAP, cIAP-1, and cIAP-2 thus preventing caspase-dependent apoptosis and consequently, cell death. People groups with the lowest incidence of pancreatic cancer have been found to have a diet high in spices such as turmeric and ginger. Curcumin is a turmeric component which has been shown to down-regulate IAPs and reduce PANC-1 cell viability. Evidence suggests that Curcumin may also trigger cell death pathways other than caspase-dependent apoptosis. We hypothesize Curcumin overcomes chemoresistance in pancreatic cancer by modulating factors involved with alternate cell death pathways: caspase-independent apoptosis and necroptosis. We investigated Curcumin-induced morphological changes characteristic of cell death via Hoffman microscopy and protein expression using SDS-PAGE and Western Blotting. This study shows Curcumin increases protein levels of Apoptosis Inhibitory Factor (AIF) and Receptor-Interacting serine/threonine-Protein kinase 1 (RIP1) at early time points in PANC-1 cells. Furthermore, AIF protein expression remains elevated, but RIP1 expression decreases by 24 hours. Curcumin's cell death modulation makes it a promising template for chemotherapeutic design.

3

Student

Postnatal maturation of neuronal morphology in the hypoglossal motor nucleus of the developing rat

Paul A. Williams and Christopher G. Wilson

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Autonomic control centers within the brainstem generate and regulate breathing rhythm. Previous studies have shown that postnatal days 10 through 13 (P10 to P13) represents a “sensitive window” for both neurotransmitter expression and changes in respiratory function. However, little is known about early developmental changes in the morphology of neurons in respiratory regions of the brainstem. Motor neurons show dramatic differences in dendritic arbor between neonates and adults. The hypoglossal motor nucleus (XII) is a region critical for the control of the tongue and coordinating airway opening with breathing. We hypothesize that specific changes occur in the morphology of neurons in the XII during early development with a sensitive period (P10 to P13). To test this hypothesis we used Golgi-Cox staining to examine the somata and dendritic arborization of developing neurons in the XII of Sprague-Dawley rat pups. At each of nine postnatal ages (P1 through P21), we removed the brain, processed the tissue, sectioned (150 micron thickness) using a vibratome, and performed Golgi-Cox staining to visualize the dendritic morphology of neurons in XII and then quantified their arbor complexity. We obtained image stacks and used 2D Sholl analysis and 3D morphometrics to quantify changes in arborization. The dendritic arbor of developing neurons became more complex from P1 to P13 as indicated by the increase in the number of radial intersections with a gradual increase from P1 to P12, then a marked increase at P13. The maximum radius at which intersections increased was approximately 200 microns (P1 to P5) to 300 microns (P7 and P10), with a further increase to ~400 microns (P12 to P13). These dendrite morphology changes appear to reflect the previously described period for neuronal development related to respiratory control. Our results suggest that morphological changes coincide with changes in neurotransmitter expression as well as respiratory function.

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Student

Recombinant Slit2 Preserves Blood Brain Barrier Integrity in Murine Intracerebral Hemorrhage

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Center for Neuroscience Research, Loma Linda University School of Medicine, Loma Linda, CA 92350

Intracerebral hemorrhage (ICH) is the least treatable and most fatal stroke subtype. Concomitant with the expansion of the hemorrhage in the brain parenchyma is significant vascular disruption, manifested as increased permeability of the blood-brain barrier (BBB). We previously found that administering recombinant (r)Slit2, a secreted chemorepellant, improved neurological outcomes after surgical brain injury. Additionally, Slit2 signaling through the roundabout receptor (Robo) 4 played a role in reducing vascular disruptions. Thus, we hypothesized that Slit2, by activating the Robo4 receptor, could mitigate the ICH-induced increase in BBB permeability and improve overall functional outcomes. ICH was induced in CD-1 mice by collagenase infusion. A time course profile (3, 6, 12, 24, and 72 hours) of Slit2 and Robo4 was developed by Western Blotting. Cellular localizations were evaluated by immunohistochemistry. Four groups of animals were utilized: sham, ICH + vehicle, ICH + 3g/kg rSlit2, ICH + 10g/kg rSlit2. rSlit2 was administered 1h after ICH induction. Brain water content (BWC) measurement and neurological tests were conducted at 24 and 72 hours after ICH. The extravasation of Evans Blue Dye was also evaluated at 24 hours.

Following ICH, the expression of endogenous Slit2 was transiently decreased, while endogenous Robo4 immediately increased and remained elevated, in comparison to sham. Robo4 was co-localized on endothelial cells. BWC and neurological deficits were significantly increased in the vehicle group and were attenuated by treatment with rSlit2 at both 24 and 72h after stroke. Administration of rSlit2 also decreased extravasation of the Evans Blue Dye.

ICH resulted in significant loss of BBB integrity. Treatment with rSlit2 restored the functionality of the BBB. Consequently, there was an improvement in functional outcomes (reduced BWC and neurodeficits). Our findings suggest that rSlit2 is a potential therapeutic candidate for intracerebral hemorrhage and possibly functions through an increase in tight junction proteins.

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Student

LHX2 is an intermediate factor in FGF-mediated SHH expression during limb development

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During limb development, wound healing, and regeneration, Fibroblast growth factors (FGFs) and Sonic hedgehog (SHH) regulate each other in a positive feedback loop. The molecular mechanisms governing this feedback loop however are poorly understood. In vertebrates with limited regenerative capacity such as chickens, FGF treatment following limb amputation during development induces a regenerative response wherein SHH and other FGF-responsive factors are re-expressed and a normal limb grows. In higher vertebrates, there is a failure to initiate an FGF signaling loop. Consequently, an absence of growth and patterning factors such as SHH and a truncated limb phenotype is achieved. Studying the molecules involved in FGF-mediated SHH expression will not only provide insights into the regulation of limb development, but also provide greater understanding regarding wound healing and redevelopment. Knowledge from the latter has the potential to enhance the wound healing process, thereby alleviating costs associated with treating individuals living with limb loss and lead to an improvement in their quality of life.

In order to identify genes responsive to FGF signaling during SHH upregulation, we performed a DNA microarray analysis. The transcription factor Lim homeobox 2 (LHX2) was upregulated >5-fold and is highly conserved across vertebrate species. Additionally, LHX2 is expressed in an FGF-responsive domain that overlaps SHH expression. It is our hypothesis that LHX2 is involved in FGF-mediated SHH expression. In situ hybridization of chicken embryos implanted with FGF beads demonstrate that LHX2 is upregulated in the presence of FGFs. By chemically blocking protein translation we observed that LHX2 is a primary response target of FGF signaling. Overexpression studies of LHX2 in the presence of FGFs result in increased expression of SHH while a knockdown of LHX2 in the limb decreases SHH expression. These data suggest that LHX2 is an intermediate factor in FGF-mediated SHH expression in the developing limb.

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Student

Glucocorticoid-Mediated Upregulation of Stress Oncoproteins: Implications for Prostate Cancer Health Disparities

Leanne Woods-Burnham, Arthur Love, Laura Stiel, Christina K. Cajigas-Du Ross, Evelyn S. Sanchez, Kwame Amponsah, Susanne Montgomery, Colwick Wilson, Carlos A. Casiano*

Loma Linda University School of Medicine, Loma Linda University School of Behavioral Health

Background: The biological characteristics of prostate cancer (PCa) tumors are exaggerated in AA men compared to European American (EA) men. There is, however, a gap in our understanding of the mechanisms underlying increased PCa aggressiveness in AA men; therefore, there is a critical need to identify biological determinants contributing to PCa health disparities.

Glucocorticoids, a type of stress hormone, have recently been implicated as biological driving factors in PCa progression. A clinical dilemma exists as glucocorticoids, important in the palliative care of PCa patients are now emerging as accelerators of disease progression. In addition, chronically elevated levels of cortisol have been documented in AA men and are linked to stressful life events; AAs are also more sensitive to glucocorticoid exposure than EAs.

Our preliminary studies have focused on examining the effects of glucocorticoids on the activation of stress oncoprotein LEDGF/p75. We have previously shown that LEDGF/p75 is upregulated in PCa tumors and our longterm goal is to explore the possible interplay between glucocorticoids and LEDGF/p75 in the context of health disparities.

Hypothesis: Glucocorticoids induce LEDGF/p75 upregulation and this may occur more robustly in AA men.

Methods and Results: (In vitro) We pharmacologically stimulated/inhibited glucocorticoid receptor (GR) using agonists/antagonists in a racially diverse panel of PCa cell lines and observed LEDGF/p75 expression with immunoblotting. Glucocorticoids upregulated LEDGF/p75 most significantly in AA cells. Conversely, mifepristone attenuated this upregulation. (Translational) ELISA assay revealed increased circulating levels of LEDGF/p75 in sera of AA men with PCa.

Conclusions and Impact: These findings imply that glucocorticoids influence the activation of LEDGF/p75 stress pathway associated with PCa aggressiveness. Completion of this innovative, high-impact project will increase understanding of the mechanisms underlying stress hormones contribution to PCa, encourage interventions to curb PCa aggressiveness in vulnerable populations, and contribute towards lessening the burden of PCa mortality in AA men.

7

Student

Synthesis and characterization of silver nanoparticles using economical and sustainable methods

**Elvin M Walemba1, Erwin Stuffle2, Claire Stewart2, Ryan G. Sinclair3, Christopher C Perry4, Danilo Boskovic4*

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Antimicrobial silver containing nanoparticles have found applications in biomedical and clinical settings. The antimicrobial properties of silver are shape, size and surface chemistry dependent. Our results show silver-gold bimetallic nanoparticles (mean diameter 16 ± 5 nm) inhibits planktonic growth of P. gingivalis, E.coli and MRSA. Based on this we expect that bimetallic silver-gold NPs (10 to 200 nm), synthesized from silver nanoparticle seeds, should produce a broad range of antimicrobial activity. Our group is able to synthesize nanoparticles between 10 and 200 nm from silver nanoparticle seeds using economical and sustainable methods. This methodology involves AgNO3 reduction by maltose, citrate, hydroquinone, ascorbic acid or polyphenylamine. The nanosilver was coated with ethylene glycol triblock copolymers and then characterized using UV-vis, DLS, and AFM. Single populations of nanoparticles were produced by varying the temperature, pH and rate of reaction. This demonstrates the facile synthesis of nanosilver of different sizes. This method is suitable for laboratories with few resources.

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Student

Impact of Oral Pathogen (Porphyromonas gingivalis) on Platelet Activation in Human Whole Blood

**William Chen1, Hansel M. Fletcher2, Melanie Thornburg3, Karen L. Hay3, Brian S. Bull3 and Danilo S. Boskovic1*

Divisions of Biochemistry1 and Microbiology2, and Department of Pathology3, School of Medicine, Loma Linda University, Loma Linda, CA 92350

During a vascular injury, the hemostatic system minimizes blood loss by forming a platelet plug at the injury site. This is accomplished by both vascular and humoral responses. The humoral response primarily comprises of the activation of platelets and a cascade of coagulation factors. Perturbations of the hemostatic system can occur as a result of exposure to a variety of triggers. Porphyromonas gingivalis is a gram negative anaerobe that possesses a wide range of virulence factors that play an important role during infection of the oral cavity while also evading host's immune response. We hypothesize that specific P. gingivalis surface/secreted components are involved in the activation of platelets.

Live P. gingivalis culture was grown to an optical density at 600 nm (OD600) of 1.0. Whole blood samples were collected from consenting volunteers. The blood was exposed to P. gingivalis for 30 minutes prior to staining for 15 minutes with an antibody cocktail and a platelet activator agonist (ADP or TRAP). Samples were fixed with 1% paraformaldehyde for 15 minutes, diluted with Phosphate-Buffered Saline (PBS), and analyzed by flow cytometry.

The ADP stimulated whole blood samples not exposed to P. gingivalis had a 29% increase in platelet activation compared to the same samples stimulated by TRAP. In the absence of P. gingivalis whole blood treated with PBS or ADP exhibited <1% or 31% overall platelet activation, respectively. In the presence of P. gingivalis whole blood treated with PBS or ADP led to 25% or 52% platelet activation, respectively.

These preliminary results suggest that P. gingivalis is able to modulate the hemostatic system through increased platelet activation in the presence or absence of other platelet activators.

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Student

The Valosin-Containing Protein Inhibits the Mitochondrial Permeability Transition Pore Opening Via Inducible Nitric Oxide Synthase in the Heart

Shaunrick Stoll, Erik Behringer, Hongyu Qiu*

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Introduction: The mitochondrial permeability transition pore (mPTP), a non-selective channel in the inner mitochondrial membrane, plays a critical role in mediating cell death during ischemia/reperfusion (I/R). We previously found that overexpressing valosin-containing protein (VCP), an ATP-binding protein, provides an inducible nitric oxide synthase (iNOS) dependent attenuation of apoptosis of cardiomyocytes and reduction of infarct size in transgenic (TG) mouse heart during I/R. Thus, we tested the hypothesis that VCP mediates cardiac protection by inhibiting the opening of the mPTP via iNOS.

Methods and Results: A cardiac specific VCP TG mouse was generated, which increased the expression of VCP by 3.5 fold and iNOS by 2.5 fold compared to WT ($P<0.05$). Cardiac mitochondria were isolated from adult VCP TG and their litter-matched wild type (WT) mice (N=4/group). MPTP opening was induced by Ca2+ overload and was measured by mitochondrial swelling using spectrophotometry. Compared to WT mice, VCP TG showed a significantly lower mitochondrial swelling ($P<0.05$), indicating mPTP inhibition. As an alternative, mitochondrial Ca2+ retention capacity was also measured using a Ca2+-sensitive probe in the presence of cumulative Ca2+ boluses (5mM). VCP TG showed a significant ($P<0.05$) reduction of fluorescence intensity versus WT, further confirming reduced mPTP opening. To test whether protection by VCP is mediated by iNOS, a bigenic VCP TG/iNOS knock out (KO-/-) mouse was generated. Deletion of iNOS from the VCP TG mice prevented the ability of VCP to inhibit the mPTP. Pharmacological treatment with cyclosporine A (CsA), a known mPTP inhibitor, blocked the mPTP in both VCP TG and WT mice, but not in VCP TG/iNOS KO-/- mice, indicating a CsA-independent mechanism.

Conclusion: We demonstrate here that VCP prevents the opening of the mPTP in an iNOS dependent manner, which may represent a novel mechanism of cardiac protection.

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Student

Sibling Recurrence Rate of Gastroschisis from a 12 Year Cohort in Southern California

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1 Department of Earth and Biological Sciences, Basic Sciences, School of Medicine, Loma Linda University, CA 2 Department of Pediatrics, Loma Linda University, Loma Linda, CA 3 Perinatal Research Institute, Loma Linda University Children's Hospital, Loma Linda, CA 4 School of Public Health, Loma Linda University, Loma Linda, CA 5 Department of OB/GYN, School of Medicine, University of California, Riverside

Background: Gastroschisis is a congenital birth defect of the abdominal wall. While the incidence of gastroschisis has increased globally in recent years, only a few familial recurrent cases have been reported. Specifically, 18 cases of sibling recurrence born to the same mother have been reported in the literature (Kohl et al., 2010). Methods: A cohort study was performed to determine the sibling recurrence rate of gastroschisis at a tertiary care medical center located in Southern California. 186 neonates with gastroschisis were delivered from 2003-present to 183 mothers. Among these mothers, 55 were multiparous and therefore included in calculating the rate of gastroschisis occurring in siblings. Primiparous women were excluded from this study. Results: Of 55 multiparous women included in the study, 3 women had pregnancies affected by recurrent gastroschisis. This represents a 5.45% sibling recurrence rate of gastroschisis in our study cohort. One patient delivered a female neonate affected by gastroschisis in 11/2009, and subsequently delivered a male neonate affected by gastroschisis in 12/2010. A second patient delivered a female neonate in 1/2006 affected by gastroschisis, a female neonate not affected by gastroschisis in 8/2006 which resulted in neonatal death, and subsequently delivered a female neonate in 9/2009 affected by gastroschisis. The third patient delivered a female neonate in 2/2011 affected by gastroschisis, and subsequently delivered a female neonate in 10/2015 affected by gastroschisis. Conclusions: Literature has failed to demonstrate hereditary factors in gastroschisis pathogenesis. However, the recurrence rate of familial gastroschisis suggests that women with a history of a prior neonate with gastroschisis may be at a higher risk than previously noted. Therefore, families with a history of gastroschisis may benefit from pre-conceptual counseling to discuss the higher risk of gastroschisis in a future pregnancy. Expectant mothers who have had a pregnancy complicated by gastroschisis should undergo early ultrasound ****Truncated - Over Word Limit****

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Student

Determination of Copper Transport Protein P62 as a Significant Contributor to Alzheimer's Disease Pathology

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The increasing prevalence for Alzheimer's Disease (AD) is a rising public health issue, as the extravagant costs of health care for affected patients is only growing. Controversy is maintained over the legacy paradigm concerning amyloid-beta being the fundamental genesis of AD. There has been a growing literature describing the onset of oxidative stress playing an important role in the pathology of the disease, particularly involving metal transport pathways. Brain tissue collected from AD affected patients has shown increased deposits of copper (Cu), providing reason to believe that the Cu transport system, particularly dynactin and its subunit p62, plays a role in the formation of this disease. We hypothesize in an AD affected patient, p62 degrades over time leading to a breakdown in Cu transport and the emergence of AD pathology, thereby enabling its possible use as a biomarker. For this study, p62 is knocked out using siRNA transfection in SH-SY5Y neuroblastoma cells. The cells will be stained with a copper-specific histological probe CTAP-2. We will then use additional fluorescent tags to look at the effect on other proteins of the copper transport system, including precinilin-1, ATP7B, tau and amyloid-beta. Human brain samples will be tested for p62 expression and checked for correlations regarding copper deposition using histological stains and immunoprecipitation. Our previous research regarding copper deposition in AD affected brains shows promise for this series of experiments.

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Student

Chimeric CTB-Insulin Vaccine Induction of Immune Tolerance via IDO1 in Human Dendritic Cells: Role of the TGF-Beta Superfamily

Grace E. Esebanmen and William H. Langridge

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Type 1 diabetes, T1D, is a major tissue specific autoimmune disease in which loss of tolerance to pancreatic islet antigens results in T cell mediated destruction of the insulin producing β cells. A chimeric fusion protein vaccine composed of the cholera toxin B subunit conjugated to the diabetes autoantigen proinsulin (CTB-INS), was shown to suppress diabetes onset in mice and induce immune tolerance in human monocyte derived DCs (moDCs), via induction of the tryptophan catabolic enzyme, indoleamine 2,-3 dioxygenase (IDO1). Here, we investigated the involvement of the TGF-β superfamily, in mediating CTB-INS induction of IDO1 in human moDCs. Real-time polymerase chain reaction (RT-PCR) analysis of CTB-INS treated moDCs showed increased levels of TGF-β1 and activin-A mRNA transcription. To examine the role of the induced cytokines in generation of IDO-expressing moDCs, we conducted cytokine neutralization studies and found that CTB-INS induced expression of IDO1 independently of endogenous TGFβ and activin-A cytokine. Contrary to previous studies in murine models, exogenous addition of TGFβ1 or activin-A cytokines to human moDCs did not activate IDO1 biosynthesis. The bioactive small molecule inhibitor, RepSox (Sigma), repressed CTB-INS-mediated IDO1 protein biosynthesis in a concentration dependent manner, indicating a different kinase pathway inhibition other than TβRI kinase. Inhibition of Smad2/3 signaling using SB431542 did not inhibit CTB-INS vaccine activation of IDO1 biosynthesis. Together, our data suggest CTB-INS activates TGF-β /activin-A-independent and Smad2/3-independent signaling, to induce IDO1 expression in human moDCs. These findings provide insight into the mechanism of vaccine action for advancement towards clinical application for autoimmune diabetes therapy.

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Student

Aryl hydrocarbon receptor agonist 5F 203 induces oxidative stress triggering DNA damage and cytoglobin up-regulation in human breast cancer cells

Leah Rowland1, Lancelot S. McLean2, Petreena Campbell1, Cheri N. Watkins1, Dain Zylstra3, Louisa H. Amis1, Lia Scott1, Crystal E. Babb1, W. Joel Livingston1, Maheswari Senthil4 and Eileen Brantley1,3

1Department of Basic Sciences, School of Medicine, 2Center for Dental Research, School of Dentistry, 3Department of Pharmaceutical and Administrative Sciences, School of Pharmacy, 4Department of Surgery, School of Medicine Loma Linda University

Breakthroughs are needed in breast cancer therapy to improve clinical outcomes. Emerging evidence suggests that tumorigenesis stems, in part, from epigenetically silenced tumor suppressor genes (TSGs) and restoring TSGs may represent a viable strategy to treat breast cancer. We previously found that aryl hydrocarbon receptor (AhR) agonist 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203) exhibits potent cytotoxicity, increases reactive oxygen species (ROS), induces DNA damage and up-regulates the expression of putative tumor suppressor gene cytoglobin in breast cancer cells. In the current study, we seek to delineate the mechanism by which 5F 203 induces DNA damage and cytoglobin expression in susceptible breast cancer cells. We found that 5F 203 activated p38 mitogen activated protein kinase (p38) and c-Jun-N terminal kinase (JNK) signaling in breast cancer cells. Pretreatment with antioxidant N-acetyl-L-cysteine or AhR inhibitor α-naphthoflavone diminished 5F 203-mediated p38 or JNK activation in a cell context dependent fashion. Pretreatment with pharmacological inhibitors of p38 or JNK suppressed 5F 203-mediated increases in intracellular ROS to suggest the presence of a positive feedback loop. 5F 203 induced oxidative DNA damage in breast cancer cells but not breast epithelial MCF-10A cells unlike AhR agonist benzo[a]pyrene which induced oxidative DNA damage more indiscriminately. Pretreatment with p38 or JNK inhibitors suppressed 5F 203-induced single strand break formation and cytoglobin mRNA expression in breast cancer cells. Our data show 5F 203 confers anticancer activity in breast cancer cells in part by increasing ROS via a positive feedback loop to sustain p38 and JNK activation resulting in DNA damage and cytoglobin restoration.

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Student

Differential Expression of Survivin Splice Variants in Chemosensitive and Chemoresistant Pancreatic Adenocarcinoma Cell Lines

Janviere Kabagwira, Amber Gonda, Jenniffer Licero, James McMullen, Nathan R. Wall

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Despite significant progress in the treatment of pancreatic cancer, chemoresistance remains a problems. The other major problem is the lack of diagnostic tools for its early detection. Pancreatic Adenocarcinoma develops resistance against many currently used chemotherapies including Gemcitabine, the first line approved chemotherapy agent. Survivin, a member of inhibitors of apoptosis (IAP) family and a cell cycle-associated oncoprotein has been associated with chemoresistance in many cancers. Additionally, dysfunction in alternative splicing “the process of removing non coding regions of a gene” has been implicated in different disease states including many cancers. We hypothesize that differential expression of survivin is associated with sensitivity and/or resistance to Gemcitabine. To test our hypothesis, we used RT- PCR to evaluate the expression of all known Survivin splice variants in Gemcitabine-resistant and Gemcitabine-sensitive cell lines, PANC1 and Mia PaCa 2 respectively. To further test our hypothesis, we plan to overexpress survivin splice variants that are found upregulated in resistant cell line in Gemcitabine-sensitive cell line to see the effect on chemoresistance. Varying expression of survivin splice isoforms in resistant and sensitive pancreatic cancer patients may serve as a prognostic tool for pancreatic cancer and help to define an appropriate therapy, matching therapeutics with appropriate individual oncotargets.

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Student

Anti-microbial silver, indicator organisms and pathogens in physician's white coats worn in an out-patient clinic.

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School of Medicine and School of Public Health

Introduction: Microbial contamination of hospital surfaces and clothing is a growing concern with an emphasis on infection control and novel antimicrobials such as silver. There have been several studies detailing the contamination of white coats, scrubs and ties in hospital health care settings. This study seeks to quantify the microbial contamination on white coats worn by physicians in a Loma Linda University outpatient clinic, and to test the hypothesis that antimicrobial silver has an inhibitory effect on contaminates.

Methods: Resident and attending physicians are recruited through a chain-referral sampling method and given a PureThread© white coat in exchange for their regular white coats. The participants wear the test coats for 2 weeks, and hand them over for testing at the LLU environmental microbiology lab. The coat is put in a stomacher, and 300ml of Buffered Peptone Water (BPW) is added. The coat is manually squeezed for three minutes and the BPW eluted from the coats, and processed for Vancomycin-Resistant Enterococci (VRE), Enterococci (Non-VRE), Methicillin-resistant Staphylococcus aureus (MRSA), Carbapenem-resistant E.coli(CRE), and Total coliform.

Results: All the 27 non-silver white coats tested were positive for Staphylococcus bacteria, 2 coats had E.coli, 21 were positive for Coliform and 17 were positive for Enterococcus. All Staphylococcus were methicillin-resistant (MRSA), but with lower concentrations. One E.coli was CRE and 10 Enterococcus were VRE. All the three PureThread coats processed had lower concentrations of MRSA that the non-treated coats, one was positive for total coliforms, and two were positive for Enterococcus with one being VRE. Overall, fewer coats were contaminated with E.coli or Total Coliforms. PureThread coats have lower contamination, and show lower concentrations of all drug-resistant bacteria.

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Student

The impact of estrogen withdrawal on the KRAS-variant in breast cancer

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Background: The KRAS-variant is associated with increased risk of lung, breast, and ovarian cancer, as well as of multiple cancers in the same patients. Recently, we have shown that estrogen withdrawal leads to enhanced transformation in KRAS-variant cells in vitro, and in an increased breast cancer risk for women with the KRAS-variant. The aim of this study was to further understand how estrogen withdrawal enhance transformation. We targeted several different receptors and mechanisms of estrogen signaling, including ER α and ER β, GPER and aromatase, using an isogenically matched normal breast epithelial line, with (MCF10aKRAS+/-; MT) versus without (MCF10aKRAS-/-; WT) the KRAS-variant.

Methods: We used soft agar assay to evaluate anchorage independent growth and transformation in isogenic cell lines with different anti-estrogen treatments, such as tamoxifen, fulvestrant, G36 and anastrozole. We also investigated the underlying molecular changes that lead to transformation in isogenic cell lines by performing qRT-PCR and Western Blotting analysis.

Results: We that both KRAS-variant and WT lines have virtually no ER α or ER β expression. However, we found that GPER, a novel estrogen transmembrane receptor, was expressed in WT and KRAS-variant lines as well. In addition, we found that KRAS-variant lines had significantly elevated aromatase and androgen receptor gene expression levels compared to WT lines. As expected, returning estrogen to the media reduced transformation. We also found that a selective GPER antagonist, G36, completely abrogated transformation. Fulvestrant, known to be a GPER agonist, also decreased transformation. However, inhibition of aromatase by treating cells with anastrozole, which likely further decreases cellular estrogen, was shown to increase transformation.

Conclusions: These findings confirm that estrogen withdrawal increases transformation in normal epithelial breast cells and show that oncogenesis with estrogen withdrawal in the presence of the KRAS-variant may involve GPER. Studies are ongoing to further investigate and validate involvement of GPER.

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Student

Rapid metabolism of plasma dinitrosyl iron complexes attenuates their vasodilatory properties

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Nitric oxide (NO) is an important signaling molecule that is exploited for its vasodilatory properties in hypertension treatment. The lability of NO compared to its long-lasting systemic effects however suggests the existence of stable storage forms. Dinitrosyl iron complexes (DNICs) are a possible candidate for NO storage molecules due to their stability and ability for controlled NO release. Endogenous DNICs occur with either low molecular weight cysteine/glutathione ligands, or high molecular weight protein ligands, the latter being more stable. Low concentrations of cellular DNICs prompts the use of exogenous DNICs to investigate their biological properties and possible involvement as an intracellular NO store. DNICs have thus been found to be potent vasorelaxants in vessel bath experiments with isolated arteries. The potent vasorelaxant properties of DNICs do not however translate to significant hypotension following intravenous infusion in vivo, as observed in our studies with sheep. This study sought to understand the reason why DNICs lose their vasodilatory properties once injected into sheep. DNICs were added to isolated sheep whole blood and plasma, and triiodide-based chemiluminescence was used to detect DNICs as well as their likely metabolites, nitrite and S-nitrosothiols (SNOs). DNICs were determined to be erythrocyte-impermeable, and were rapidly but only partially degraded to nitrite in both sheep blood and plasma. The remaining DNICs are stable and resist NO release. The stable species are most likely high molecular weight DNICs that formed due to exchange of the original glutathione ligands with albumin thiolate/histidine ligands; Both protein stabilization and degradation of DNICs to the vaso-inactive nitrite might explain their loss of vasodilatory properties upon injection into sheep. These results help to understand how the potent in vitro vasodilatory effects of DNICs are lost in vivo, and can guide the current development of DNICs into pharmacological agents to treat hypertension.

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Student

Enhanced Chondrogenic Potential of Peripheral Blood Cell-Reprogrammed Mesenchymal Stem Cells via SOX trio Overexpression

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Mesenchymal stem cells (MSCs) hold promise for cartilage regeneration. However, current MSC-based approaches in clinical and preclinical settings for joint repair face two major problems: (1) availability of autologous cell source, and (2) quality of cells. MSCs harvested from marrow or adipose tissue, especially from older patients, only showed limited chondrocytic potential after in vitro expansion. Recently, we have successfully converted adult peripheral blood cells, a noninvasive and readily accessible cell source, into induced mesenchymal stem cells (PB-iMSCs) using clinically relevant non-integrating vectors. Rejuvenated by the reprogramming process, these PB-iMSCs have greater chondrogenic potential than marrow MSCs, which provides a prominent autologous cell source for joint repair.

The aim of this study is to further improve the chondrogenic potential and functional quality of neocartilage generated from PB-iMSCs. We hypothesize that overexpression of SOX trio (SOX 5, 6, 9) will enhance the in vitro chondrogenic differentiation of PB-iMSCs, and improve the elastic modulus of generated cartilage tissue. The SOX trio plays a vital role in chondrogenesis during embryonic development, and it also maintains the chondrogenic phenotype to promote cartilage regeneration. We transduced PB-iMSCs with lentiviral SOX trio or control GFP. After culturing cells in chondrogenic medium for three weeks, RT-qPCR analysis showed that SOX trio overexpression leads to a 50-fold increase in the expression of COL2A1 and ACAN, articular chondrocyte specific genes. In addition, the expression of hypertrophic marker COL10A1 was significantly reduced by 20-fold. These data demonstrate the articular chondrocyte nature of the generated cells.

To evaluate the functionality of PB-iMSCs generated articular neocartilage, we seeded 2 x 106 SOX trio or control GFP transduced PB-iMSCs in the fibronectin coated electrospun scaffold. After culturing in chondrogenic medium on an orbital shaker at 100 rpm for 7 weeks, we found that the elastic modulus and viscoelastic modulus of the SOX trio ****Truncated - Over Word Limit****

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Student

Epigenetic Regulation of Osteoblast Differentiation by Vitamin C Involving Prolyl Hydroxylase Domain-containing Protein 2 (PHD2)

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Osteoporosis is a devastating and common chronic disease characterized by low bone mass and quality leading to increased fragility and risk of fracture. Osteoporosis occurs when bone resorption by osteoclasts is not compensated by bone formation by osteoblasts (OBs). Vitamin C (ascorbic acid, AA) is a critical regulator of OB differentiation, and our laboratory has established that PHD2 plays a role in mediating this effect. Additionally, recent studies have shown AA is involved in epigenetic regulation of gene expression, inducing demethylation of many gene promoters in embryonic stem cells by activating ten eleven translocase (TET)-mediated conversion of 5-methylcytosine to 5-hydroxymethylcytosine (5-hmC). Since TETs and PHDs belong to the same family of 2-oxoglutarate-dependent dioxygenases which require AA for their activities, we hypothesized that the biological effect of AA in OBs may in part be due to PHD2-mediated promoter demethylation of genes involved in the OB differentiation program. To test this hypothesis, we cultured MC3T3-E1 pre-OBs and primary mouse OBs with AA and measured the amount of 5-hmC by dot-blot and ELISA. AA-treated OBs contained significantly higher levels of 5-hmC. Next, to establish whether PHD2 could mediate this AA-induced DNA demethylation, we evaluated PHD2 nuclear localization by immunoblotting and found significant nuclear localization of. Consistent with our hypothesis that PHD2 mediates gene promoter demethylation, the ability of AA to increase 5-hmC was blocked in OBs treated with PHD2 inhibitor IOX2. Finally, we evaluated whether AA induced demethylation of genes involved in OB differentiation, using a 5-hmC-specific antibody to enrich demethylated DNA. We found increased levels of demethylation in known AA target genes Osx, Alp, and Ihh. Together, our findings suggest that AA promotes transcription of target genes in OBs in part via epigenetic modification of DNA by a PHD2-mediated mechanism. This mechanism should be exploited towards development of anabolic therapies for osteoporosis.

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Student

Functional Contexts And Roles Of Fatty Acid Binding Proteins 4 And 5 In Rats Following Spinal Cord Injury

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The pathophysiology of spinal cord injury (SCI) results from both a causative mechanical insult and subsequent resolution of injury. Neuronal cell death and membrane rupture, occurring at injury onset, are responsible for shifts in the spinal cord lipidome known to promote differentiation of resident microglia and infiltration of peripheral inflammatory cells. Our lab and others have documented an increase in pro-inflammatory lipids, and in the Ï‰-6: Ï‰-3 polyunsaturated fatty acid (PUFA) ratio in injured rat spinal cord epicenters when compared to controls. Pro-inflammatory fatty acids, including Ï‰-6, are known to promote death of neuronal cell populations and diminish axonal reconnection. Consequently, investigating proteins involved in binding and shuttling of fatty acids in neuronal and inflammatory cells is imperative to the development of therapies for locomotor and sensory loss following SCI. Fatty acid binding proteins, particularly fatty acid binding protein 4(FABP4) and 5 (FABP5), are good therapeutic targets as they bind pro- and anti-inflammatory lipids respectively. Our previous publications have documented the presence of FABP5 in neurons, glia, oligodendrocytes, astrocytes, and neural progenitors and its ability to promote neuronal survival and locomotor recovery through binding docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA) in expressing cells. Furthermore, inhibition of FABP5 was shown to hinder locomotor recovery post SCI. In contrast, unpublished data from our lab suggests that FABP4, whose expression is prevalent in monocytes and macrophages, plays an opposing role. Studies looking at mRNA and protein levels of FABP4 revealed a 100-fold mRNA and 15-fold protein increase in injured rats at 7 days post injury (dpi) compared to controls. Furthermore, inhibition of FABP4 improved locomotor recovery scores at 1,3,7,12, and 14 dpi. Because of these distinct expression profiles and functional contexts, we hypothesize that modulation of FABP5 and FABP4 expression after injury is essential in promoting locomotor and sensory recovery after SCI.

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Student

Mild Pediatric Traumatic Brain Injury Elicits Long-Term Oligodendrocyte-Associated White Matter Dysregulation

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Mild traumatic brain injury (mTBI) is a major public health concern often accompanied by long-term behavioral and neuropsychological deficits. Emerging data suggest that these deficits are exacerbated following repeated injuries. However, unlike adult mTBI, the effects of mTBI on white matter structure and myelination process in young children have not been extensively examined. Moreover, the effect of repeated mTBI on developing white matter has not been studied. To address this knowledge gap, we investigated the long-term effects of single and repeated pediatric mTBI on white matter tracts, focusing on the anterior commissure (AC), using diffusion tensor imaging (DTI) and immunohistochemistry (IHC). We hypothesized that repeated mTBI to the developing mouse brain leads to abnormality in microstructural integrity and impaired oligodendrocyte development. mTBI was induced at postnatal day 14 using a closed head injury (CHI) model. Sham, single mTBI, and repeated mTBI (3 days after the initial injury) were compared. After CHI the mice were evaluated at 60 days post injury (DPI) utilizing DTI and IHC. We observed that mTBI at 60DPI resulted in higher fractional anisotropy (FA) indicating diffusion asymmetry and lower radial diffusivity (RD) indicating structural myelin change in the AC for both single and repeated groups. We also examined transcription factors crucial to oligodendrocyte differentiation: Oligodendrocyte transcription factor (OLIG2) and adenomatous polyposis coli (APC). Olig2, a marker of oligodendrocyte progenitor cells (OPC), showed decreased levels in the AC after smTBI, whereas APC, a marker of mature oligodendrocytes, showed increased levels after rmTBI. The observed changes in FA and RD confirm the presence of dysregulation of the white matter microstructure. Moreover, the IHC results indicate a possible pathway related to abnormal oligodendrocyte development. We conclude that repeated mTBI to the developing mouse brain elicits oligodendrocyte development dysregulation which is associated to long-term white matter structural abnormalities.

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Student

Colorectal Cancer Metastatic Associated Proteins In Serum Exosomes

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Peritoneal Carcinomatosis (PC) is defined as epithelial cancer growths along multiple regions of the peritoneal surface. PC often originates from visceral organ metastasis. One of the most common cancers that metastasizes to the peritoneum is colorectal cancer (CRC). CRC can also metastasize to the lungs and liver. The mechanism behind CRC metastasis to specific organs is unknown. Exosomes from patient's blood were examined in order to study factors related to specific organ metastasis in a manner that yielded clinically applicable data. Exosomes, 30-150 nm lipid bound vesicles, that are released by cells, contain multiple biomarkers, including proteins, which reflect internal cellular processes. Exosomes protect their contained proteins from degradation by external enzymes. This makes blood born exosomes ideal for diagnostic purposes. To analyze specific organ metastasis, proteins from serum exosomes were examined from pooled patient sample groups: PC metastatic CRC patients, liver metastatic CRC patients, and CRC patients without metastasis. Exosomes from the respective grouped patient's sera were concentrated with ExoQuickTM then lysed. Contained proteins were digested with sequencing grade Trypsin and examined with a ThermoFisher Q Exactive Mass Spectrometer. The resultant peptide sequences were compared to known protein sequence data in the SwissProt Human protein database. The data is yielding a panel of proteins that may prove useful for screening for metastatic recurrence post curative CRC surgery and will yield data about the cancer's effects in the body.

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Student

Exosomal Survivin associates with endocytosis receptors

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The tumor microenvironment is a crucial player in tumorigenesis, proliferation, and survival. Communication through vesicular trafficking is central to the interaction between tumor cells and the surrounding milieu. Exosomes, nano-sized vesicles, carry various proteins and nucleic acids to other cells, both locally and systemically. Those exosomes derived from tumor cells contain proteins and nucleic acids essential to the development and maintenance of tumors. One such protein, Survivin, a member of the Inhibitor of Apoptosis (IAP) protein family, has been identified in all cancer cells and correlates with poor prognoses. Intracellular Survivin plays a key role in cell division and cell cycle regulation as well as in the inhibition of apoptosis. Recent research has identified a unique pool of Survivin in the extracellular environment, where it contributes to a more aggressive phenotype in recipient cancer cells.

Using tandem affinity purification and immunoprecipitation we have identified a relationship between Survivin and the TNF receptor and transferrin receptor families. These endocytic receptors have been noted to be upregulated in cancer cells. We therefore hypothesize that exosomal Survivin is taken up by adjacent cancer cells via receptor-mediated endocytosis. We have shown that in the presence of antibodies to these receptors, there is a decreased uptake of extracellular Survivin. Results were similar when conditioned media was introduced to HeLaS cells with siRNA knockdowns of each receptor, as well as to CHO cells deficient in these receptors. We conclude that uptake of extracellular Survivin into cancer cells is dependent upon these two endocytosis receptors. Understanding this mechanism of signaling between cancer cells shows the potential that intercellular trafficking of oncoproteins plays in tumor maintenance and progression. It also provides us with a novel means to combat the resulting enhanced aggressive nature of the disease and sheds light on potential mechanisms of metastasis.

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Student

Exo-Proteomic Profiles of Patients With Traumatic Brain Injury

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INTRODUCTION: Traumatic Brain Injury is a leading cause of disability in the United States. According to estimates from the Centers For Disease Control, at least 1.7 million TBIs occur in the United States every year. This figure does not account for the numerous incidences of unreported closed head traumas. Additionally, traditional diagnostic parameters for TBIs are limited and there remains a significant need for better diagnostic tools. Discovery of trauma-specific biomarkers would significantly aid in the early detection and possible treatment of TBIs.

METHODS: Serum samples were obtained from patients admitted to the Trauma Department at the Level One Trauma Center of Loma Linda University Hospital for various traumas. Patients were evaluated according to the Glasgow Coma Scale (GCS) score at admission and 48 hours after admission. 27 Patient samples were categorized based upon their respective GCS scores and stratified into the following groups: no head trauma (GCS: 15), mild/moderate head trauma (GCS: 9-14), and those exhibiting severe trauma (GCS:3-8). Exosomes were isolated via the use of Exoquick, a proprietary exosome isolation technique developed by System Biosciences Inc. and their protein contents were analyzed via liquid chromatography mass spectrometry (LC/MS/MS). Protein identification was performed via SeaQuest software and protein characterizations were undertaken with the aid of Uniprot and NBCI protein databases.

RESULTS: Mass spectrometry analysis yielded over 8,000 protein hits of which approximately 600 were both patient and/or group specific. Patients with similar injury mechanisms and severity of presentation also exhibited differential protein expression.

CONCLUSIONS: Our efforts to date have elucidated a compendium of serum proteins and we continue our efforts to identify a GCS category dependent and clinically relevant biomarker are currently underway in our laboratories as we evaluate our controls and three GCS stratified groups for exosomes.

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Student

Formation of Platelet-Neutrophil Aggregate (PNA) in the newborn is Age Dependent.

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Platelets recruit neutrophils to an injury site leading to inflammatory response and clearance of extracellular pathogens. There is a lack of data on this interaction in the newborn. We hypothesize that platelets from term newborns will interact more readily with neutrophils than platelets from preterm neonates. “Lyse-no-wash” flow cytometry of cord blood samples was done on Miltenyi MacsQuant flow cytometer. Samples were collected shortly after delivery from 42 neonates born at the Loma Linda University Children’s Hospital (LLUCH). After incubation with monoclonal antibodies and the agonists ADP or TRAP (thrombin receptor activating peptide), platelet positive neutrophils were identified by the presence of CD61 PerCP on neutrophils. Level of platelet activation (P-Selectin expression) was also determined by evaluating the percentage of CD62P PE (P-selectin) expressed on platelets. Flow cytometry data were analyzed on Flowjo software. One-way analysis of variance (ANOVA) and Pearson correlation were used to analyze the data. The study was approved by the IRB and informed consent obtained from the mothers. The neonates were grouped into three categories based on their gestation age at birth: Preterm (<34 weeks), late preterm (34 to <37 weeks) and term (â‰¥37 weeks). Our results show significant differences in the preterm and term newborns in the percentage of PNA formed in unstimulated (p=.033), ADP (p=.025) or TRAP (p=.004) stimulated whole blood. The late preterm newborns had values that were intermediate between the preterm and term newborns. The values for P-Selectin (CD62P) were not significantly different between the groups, however, formation of PNA correlated positively with this marker. Poor PNA formation at birth in preterm newborns may be associated with some of the morbidities experienced by this cohort in the first few days of life.

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Student

Small molecules enhance the effects of proton irradiation on glioblastoma cell line (U-138)

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Glioblastomas are radioresistant, chemoresistant tumors which are almost always lethal. Duocarmycin-SA (DSA), a small molecule DNA alkylating agent with extreme cytotoxicity has not been widely used in cancer therapy. We propose the administration of a nanomolar concentration of DSA combined with proton radiation to cause a combined cytotoxic effect that would maximize the dose to the target and minimize the dose to the surrounding tissues for the treatment of glioblastoma tumors.

To define exposure level-response relationships for protons and DSA, we treated glioblastoma (GBM) cells and normal human lung epithelial (HLE) cell with graded dose/concentrations of 250 MeV protons and DSA. Cell toxicity was evaluated by trypan blue exclusion assays and cell counting at 72 hrs. after treatment. To test the hypothesis of synergistic effects between DSA and protons we performed combined treatments using the same endpoints.

Results of our cytotoxic assay indicate that single 2 and 3 Gray doses of proton radiation alone are insufficient to effect GBM and HLE cell death respectively. Exposure to radiation significantly increases the effectiveness of a 0.1nM dose of DSA, decreasing the survival from 76% to 62% in GBM cells. This synergy continues at 0.5 and 1.0nM DSA. At higher doses of proton irradiation, the cumulative effect continues on GBM cells in the presence of DSA. At a single 3Gy dose of proton irradiation GBM cells have 62% survival. In combination with 0.1nM DSA, the survival drops to 31%. HLE cells exposed to 3Gy protons and 0.1nM DSA survived at a greater fraction than GBM cells at 82%.

These preliminary results support the hypothesis that DSA at sub-nanomolar concentrations can effectively enhance the radiosensitivity of GBM cells. The differential response of these two cell lines (GBM vs. HLE cells) to the action of DSA at low concentrations is promising from a toxicity standpoint.

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Student

Measuring the cellular response of macrophages to soil components by flow cytometry: Determining the toxicity of podoconiosis-associated soils

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LLU Basic Sciences and Earth and Biological Sciences

Podoconiosis is a disease characterized by lymphedema of the feet and legs that affects a large population in Ethiopia. It has been suggested that silica particles in associated soils may enter the lymphatic system through the feet causing immune-related obstruction. Although silica has been suggested, no specific mineral or mineral properties have been confirmed and no rationale for why such a common mineral should be so virulent in these particular locations.

Our investigation employed two approaches. The first, focused on comparing soil mineralogy, texture and composition from podoconiosis-associated regions to unaffected regions. The high clay content of soils in disease-associated regions has been referenced, as its small particle size would allow it to easily enter the lymphatic system. Statistical analysis of the particle size, elemental, and mineralogical data shows no significant difference in size or composition between podoconiosis-associated soils and unassociated soils. Further analysis of bulk chemistry, as well as clay x-ray diffraction, is in process.

The second arm focused on developing flow cytometry to quantify the immune response of macrophage cells to various minerals and soil particles. Our protocol confirmed previous reports that silica (5 um) is toxic to macrophages (MHS; CRL 2019) after 24 hours of exposure with an LD50 of ~ 2 particles/cell, while latex beads (4.5 um) showed little effect on the cells even at high doses (33 particles/cell). Podoconiosis-associated soils show a toxicity that appears similar to silica. However, the mechanism for cell death seems to be quite different. Using weight/volume (mg/mL) rather than particles/cell, the toxicity (LD50) for podoconiosis-associated and unassociated soils are being compared to silica, kaolinite and latex beads to determine the relative soil toxicity. Further flow cytometry studies are planned to evaluate macrophage activation and fibrogenic ligand production.

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Student

Western High-fat Diet Consumption During Adolescence Increases Susceptibility to Traumatic Stress while Selectively Disrupting Hippocampal and Ventricular Volumes

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Psychological trauma and obesity co-occur frequently and have been identified as major risk factors for psychiatric disorders. Surprisingly, preclinical studies examining how obesity disrupts the ability of the brain to cope with psychological trauma are lacking. The objective of this study was to determine whether an obesogenic Western-like highfat diet (WD) predisposes rats to posttraumatic stress responsivity. Adolescent Lewis rats (postnatal day, PND, 28) were fed ad libitum for eight weeks with either the experimental WD diet (41.4% kcal from fat) or the control diet (16.5 % kcal from fat). We modeled psychological trauma by exposing young adult rats to a cat odor threat. The elevated plus maze and the open field test revealed increased psychological trauma-induced anxiety-like behaviors in the rats that consumed the WD when compared to control animals one-week posttraumatic stress ($p < 0.05$). Magnetic resonance imaging showed significant hippocampal atrophy (20% reduction) and lateral ventricular enlargement (50% increase) in the animals fed the WD when compared to controls. These volumetric abnormalities were associated with behavioral indices of anxiety, increased leptin and FKBP51 protein levels, and reduced hippocampal blood vessel density. We found asymmetric structural vulnerabilities to the WD, particularly the ventral and left hippocampus and lateral ventricle. This study highlights how WD consumption during adolescence impacts key substrates implicated in posttraumatic stress disorder (PTSD). Understanding how consumption of a WD affects the developmental trajectories of the stress neurocircuitry is critical, as stress susceptibility imposes a marked vulnerability to neuropsychiatric disorders.

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Student

Stemness gene Alpha 6 integrin mediates Tamoxifen resistance in ER+ breast cancers

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De novo and acquired resistance to the agent tamoxifen (TAM), commonly used to treat estrogen receptor positive (ER+) breast cancer, have significantly diminished its clinical efficacy. Breast tumor-initiating cells (TICs) function as key contributors to resistance, owing to their ability to evade treatment and self-renew. Alpha 6 (α6) integrin promotes TIC capacity and survival via pathways associated with TAM resistance. α6 exist as two cytoplasmic domain variants, α6vA/B (α6 variant A/B). Previous studies indicate an association between α6vB expression and TIC potential. TAM treatment has been shown to increase TIC properties in mammary tumors, promote mammosphere formation (an in vitro model of enriching TICs) and increase α6 integrin expression. In contrast, anti-tumor aryl hydrocarbon receptor (AhR) agonist aminoflavone (AF) opposes mammosphere formation and reduces α6 integrin expression. Consequently, we hypothesize that α6 integrin overexpression confers TAM resistance and suppressing α6 integrin expression would therefore counteract TAM resistance. Quantitative reverse transcriptase PCR (qRT-PCR) showed elevated basal α6 integrin expression in both luminal A (with acquired TAM resistance) and luminal B (with de novo TAM resistance) ER+ cell lines. The Alamar Blue assay revealed that TamR cells exhibited sensitivity to AF. Semi-quantitative RT-PCR indicated α6vB overexpression in TamR cells and the ability of AF to reduce both α6vA and α6vB expression in TamR and parental cells. Anti- α6 integrin blocking antibody NKI-GoH3 sensitized TamR cells to the active TAM metabolite 4-hydroxy-tamoxifen and enhanced AF efficacy in these cells. These findings suggest α6 integrin behaves as a novel mediator of TAM resistance and the therapeutic potential of anticancer AhR agonists, such as AF, to effectively counteract such resistance. This project is innovative as it uniquely seeks to evaluate the ability of AhR agonists to reverse TAM resistance. This is significant since combating TAM resistance is expected to decrease breast cancer related mortality.

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Student

Optimization of HPV+ tumor xenograft model to determine antitumor efficacy of spinacine

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High-risk human papillomaviruses (HPV) are the causative agent of virtually all cases of cervical cancer, up to 80% of head and neck (HN), and 40% of vaginal, penile, and vulvar cancer. Current treatment options are limited, and focus on physically removing the cancer through surgery. Chemo- and radiotherapy are commonly administered in combination or after a relapse, but these therapies are relatively ineffective. Despite the development of preventive vaccines, there remains an urgent need to develop more efficient treatment options for patients already affected with HPV, as well as the increasing cohort of immune-compromised patients. The ineffectiveness of chemo- or radiotherapy is largely a consequence of the viral oncogene E6, which increases resistance by disrupting apoptotic pathways. To inactivate E6 and restore apoptosis, we screened two commercially available libraries and identified spinacine, an E6 inhibitor, as a potential small molecule drug. The next phase of drug discovery, pre-clinical lead validation, is to determine spinacine's antitumor efficacy in vivo. To accomplish this, we developed an optimized xenograft model for HPV+ cervical and HN carcinomas. For the cervical cancer model, we selected SiHa cells; while for the HN cancer model, the fastest growing cell line UM-SCC47 was selected after screening 6 different HPV+ HN cell lines for growth in mouse xenografts. To generate more consistent tumor growth, we tested Matrigel and found that its inclusion was helpful. Moreover, to increase tumor visualization, we created SiHa cells expressing pLuc, a state-of-the-art luciferase technology, and are currently in the process of developing luciferase-expressing UM-SCC47. We also tested spinacine for toxicity in mice, and concluded that the small molecule is not toxic to mice at doses up to 20 mg/kg. Overall, we have developed an optimized xenograft model of HPV+ cervical and HN carcinoma with which to determine spinacine's antitumor efficacy.

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Student

Taxane-Sensitive And -Resistant Prostate Cancer Cells Exhibit Differences In Their Migration Potential And Transcriptome Profile

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Prostate cancer (PCa) is the most commonly diagnosed cancer in American men and the second leading cause of male cancer deaths. PCa patients undergoing treatment often develop metastasis and castration-resistant prostate cancer (mCRPC). Our group has shown that expression of lens epithelium-derived growth factor protein of 75 kD (LEDGF/p75) is elevated in PCa cells and tissues. LEDGF/p75 is a stress transcription co-activator that protects PCa cells against death induced by chemotherapeutic agents such as the taxane drug Docetaxel (DTX). DTX is the current standard of care for patients with mCRPC; unfortunately, disease progression and chemoresistance occurs in DTX-treated patients. DTX resistance is characteristic of metastatic tumors, leading to high patient mortality. We have shown previously that targeting LEDGF/75 with small molecule inhibitors or siRNA-mediated knockdown partially re-sensitized taxane resistant PCa cells to DTX treatment. Our group and others have also established a role for LEDGF/p75 in promoting increased cancer cell proliferation and clonogenicity, leading to enhanced cell survival. In this study, we aimed to examine the migration potential of taxane-sensitive (low LEDGF/p75 expression) and resistant (high LEDGF/p75 expression) PCa cells using an in vitro wound-healing assay. Taxane-resistant PCa cells showed decreased migration compared to taxane-sensitive cells. However, the opposite effect was observed in taxane-resistant cells after LEDGF/p75 knockdown, suggesting that this protein may influence cell migration. In order to gain insights into molecular mechanisms underlying taxane resistance in these cells, we performed RNA-sequencing comparing transcriptome profiles in taxane-sensitive and resistant PCa cells. Preliminary Ingenuity Pathway Analysis (IPA) of RNA sequencing data identified downregulated genes associated with cellular movement and migration in taxane-resistant PCa cells, consistent with results observed in the migration studies. Taken together, these results suggest that differences in gene expression between taxane-sensitive and resistant PCa cells may influence the migration and metastatic potential of these cells.

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Student

Small molecules enhance the effects of proton irradiation on glioblastoma cell line (U-138)

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Glioblastomas are radioresistant, chemoresistant tumors which are almost always lethal. Duocarmycin-SA (DSA), a small molecule DNA alkylating agent with extreme cytotoxicity has not been widely used in cancer therapy. We propose the administration of a nanomolar concentration of DSA combined with proton radiation to cause a combined cytotoxic effect that would maximize the dose to the target and minimize the dose to the surrounding tissues for the treatment of glioblastoma tumors.

To define exposure level-response relationships for protons and DSA, we treated glioblastoma (GBM) cells and normal human lung epithelial (HLE) cell with graded dose/concentrations of 250 MeV protons and DSA. Cell toxicity was evaluated by trypan blue exclusion assays and cell counting at 72 hrs. after treatment. To test the hypothesis of synergistic effects between DSA and protons we performed combined treatments using the same endpoints.

Results of our cytotoxic assay indicate that single 2 and 3 Gray doses of proton radiation alone are insufficient to effect GBM and HLE cell death respectively. Exposure to radiation significantly increases the effectiveness of a 0.1nM dose of DSA, decreasing the survival from 76% to 62% in GBM cells. This synergy continues at 0.5 and 1.0nM DSA. At higher doses of proton irradiation, the cumulative effect continues on GBM cells in the presence of DSA. At a single 3Gy dose of proton irradiation GBM cells have 62% survival. In combination with 0.1nM DSA, the survival drops to 31%. HLE cells exposed to 3Gy protons and 0.1nM DSA survived at a greater fraction than GBM cells at 82%.

These preliminary results support the hypothesis that DSA at sub-nanomolar concentrations can effectively enhance the radiosensitivity of GBM cells. The differential response of these two cell lines (GBM vs. HLE cells) to the action of DSA at low concentrations is promising from a toxicity standpoint.

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Student

Sinoatrial node development from primordial cardiovascular progenitor cells

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The cardiac pacemaker has been used to treat arrhythmias that arise from dysfunction of the sinoatrial node (SAN), which generates the pacing activity of the heart. Though these pacemakers are limited in responsiveness to autonomic regulation and require routine maintenance, no alternative therapies exist. Primordial Islet-1+ cardiovascular progenitor cells (CPCs) differentiate into functional pacemaker cells during development. Transcription factors from the T-box family, including Tbx3, Tbx5, and Tbx18, are implicated in this process. Induction of Tbx transcription factors, possibly through Smad-independent angiotensin II receptor type I activation, may differentiate Islet-1+ human neonatal-derived CPCs into functional SAN cells that express the characteristic funny current-carrying hyperpolarization-activated cyclic nucleotide gated (Hcn4) channel. We tested this hypothesis by treating primordial CPCs with angiotensin II for 72 hours and assessing induction of the gene expression pathway associated with SAN development. RT-PCR indicated that TBX3, TBX5, and TBX18 were induced by 2.6-fold ($P<0.01$, $n=3$), 1.45-fold, and 1.9 fold ($P<0.001$, $n=3$), respectively. This resulted in a 6-fold increase in HCN4 expression ($P<0.001$, $n=3$) and a 5-fold increase in Hcn4 protein induction, as indicated by flow cytometry. Furthermore, cardiomyocyte markers TNNT2 and NKX2-5 decreased in expression by 2-fold ($P<0.05$, $n=3$) and 7-fold ($P<0.001$, $n=3$), respectively. Treated primordial CPCs were then placed onto a polysaccharide polymer scaffold to facilitate their functional organization into a pacing patch. Scaffold-cultured CPCs experienced a 66-fold ($P<0.001$, $n=3$) increase in TBX3 expression and a 99-fold ($P< 0.01$, $n=3$) increase in HCN4 expression. Scaffold-cultured primordial CPCs were stained with Fluo4-AM, an intracellular calcium dye, which indicated the presence of auto-rhythmic calcium flux. We demonstrate that angiotensin II treatment induces a gene expression pathway associated with SAN development. Further work will characterize the electrophysiological properties of these cells. In doing so, we will develop a sinoatrial node from CPCs that can be studied in vivo.

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Student

The role of Snail in cell differentiation status: epithelial-mesenchymal transition and cancer stem cells.

The role of Snail in cell differentiation status: epithelial-mesenchymal transition and cancer stem cells.

Loma Linda University, Basic Science Department

Purpose: The epithelial-mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and become mesenchymal cells with migratory and invasive properties. EMT is essential for numerous developmental processes such as gastrulation, and is involved in the initiation of metastasis for cancer progression.

Cancer cells that undergo EMT are enabled to metastasize to form secondary tumors. One characteristic of advanced tumors is a low expression level of let-7, a miRNA known to be vital in both maintenances of cellular differentiation and tumor suppression. There is a gap in the literature concerning the mechanism of let-7 downregulation. Our purpose in this study is to present data that shows Snail, an EMT inducer, directly represses let-7, hence inducing cancer stem cells.

Experimental Procedures: Ovarian, pancreatic and breast cancer cell lines treated with TGFβ or EGF, inducers of EMT, were studied in our experiments. Expression of several markers at protein level was visualized with immunofluorescence and quantified with flow cytometry. Gene expression of mesenchymal, epithelial and pluripotency factors and miRNAs was detected by q-RT-PCR. Chromatin immunoprecipitation of Snail was performed in ovarian cancer lines. Let-7i promoter luciferase with and without Snail were co-transfected into OVSAHO and 293T cell lines and followed by luciferase assays for detection of bioluminescence. Snail was knocked down by lentiviral small hairpin RNA. Snail was overexpressed by estrogen receptor fusion protein. Orthotopic xenografts of patient-derived ovarian cancer cells were used to test the effect of Snail knockdown on tumor burden.

Results: In selected ovarian cancer lines, levels of Snail expression correlated directly with Nanog, and indirectly with let-7 expression. Snail bound promoters of let-7 in cell lines tested. Luciferase assays demonstrated direct repression of let-7 transcription by Snail. Metastatic burden was significantly reduced in cell line xenografts in which Snail was knocked down.****Truncated - Over Word Limit****

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Student

Overexpression of BI-1 will attenuate ER stress induced apoptosis and inflammation in neonatal HI rat model

Desislava Doycheva, Harpreet Kaur, Jay Malaguit

Neonatal hypoxia ischemia (HI) is an injury to the neonatal brain caused by interrupted blood flow. It occurs in 2-4 of 1000 full-term births and 60% of premature infants. It is leading cause of mortality and morbidity associated with life-long neurological impairments.

Endoplasmic reticulum stress (ER) is a major pathology encountered after HI, associated with dysregulation of protein folding leading to apoptosis and inflammation. HI induced ER stress up regulates the pro-apoptotic Inositol requiring enzyme-1 alpha (IRE1α) signaling pathway and is also associated with reactive oxygen species (ROS) accumulation, mainly from the NADPH-dependent cytochrome P450 reductase (NPR) and P450 2E1 (CYP) complex.

Bax-inhibitor 1 (BI-1) protein, expressed on ER membrane, has been shown to play a major role in inhibiting ER stress induced signaling pathways. BI-1 can directly bind to IRE1α thus inhibiting this pro-apoptotic pathway as well as reduce ROS accumulation by dissociating the NPR-CYP complex.

The objective of this study is to establish BI-1s anti-apoptotic and anti-inflammatory effects in an in vitro oxygen glucose deprivation (OGD) model and in an in vivo neonatal HI rat model as well as to elucidate the mechanisms via which it confers its protective properties. Our central hypothesis is that (1) transfection of cells with Ad-TMBIM6 vector will improve cell viability after OGD as we as help determine BI-s-1s major pathways (2) overexpression of the BI-1 protein in the brain, via Ad-TMBIM6 injection will improve recovery after neonatal HI by reducing ER stress induced (a) neuronal apoptosis via inhibition of IRE1α signaling pathway and (b) neuroinflammation via dissociation of the NPR-CYP complex and subsequent inhibition of ROS.

The long-term goals of this proposal are to: 1) establish BI-1 as main regulator of ER stress 2) establish BI-1s signaling pathways after neonatal HI; 3) provide a basis for BI-1 as a potential therapeutic target.

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Postdoctoral Scholar

Unique regeneration mechanism to restore bone after dietary calcium deficiency

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LLU and LLVAH

Dietary calcium deficiency causes a large increase in bone resorption and bone loss to compensate for the calcium deficiency. Once calcium is replenished, there is a rapid stimulation of bone formation, leading to complete restoration of the bone lost during calcium deficient. This adaptive process is known as “bone repletion”. Understanding the anabolic process of bone repletion that is capable of rejuvenating the skeleton could allow us to gain insights into the mechanism for rejuvenating the skeleton in osteoporosis and other aspects of skeletal maintenance. Accordingly, this study was undertaken to determine the mechanism of the bone repletion process. To initiate calcium depletion, we fed weanling mice a low calcium (0.01%) diet for 14 days. Calcium depletion decreased plasma calcium and increased plasma PTH (by 400%), 1,25(OH)2D, (by 300%) and CTX (a bone resorption marker) (by 250%), as anticipated. These plasma parameters quickly returned toward normal upon calcium repletion through feeding the animals a calcium-sufficient (1.2%) diet for 14 days. ÂµCT analysis revealed that trabecular bone volume, number, and connectivity decreased by -42%, -50%, -50%, respectively, at the end of the calcium depletion. Remarkably, the trabecular BV/TV, number, and connectivity were each restored to the respective control values at the end of 14 days of calcium repletion. The most salient histological finding is that there was the accumulation of multiple layers of osteoblasts and deposition of matrix proteins near the resorbing osteoclasts. The number of osteoblasts per osteoblast surface and osteoid width, surface, and volume each significantly increased by 40-100%. In conclusion, these findings suggest a paradigm-shifting concept that bone tissue exhibits a unique renewal mechanism whereby the number of osteoblasts required to replace the bone lost during calcium depletion are created and stockpiled during calcium depletion to be rapidly deployed later during a period of calcium sufficiency.

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Postdoctoral Scholar

CD105+MSCs/osteoprogenitors provide a cellular source necessary for bone repair

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Division of Regenerative Medicine

Under conditions of insufficient calcium intakes, calcium is released from bone to maintain calcium homeostasis that leads to bone loss. Upon replenishment with sufficient dietary calcium, the calcium deficiency-induced bone loss is fully restored. This self-induced bone "regeneration" process is known as "bone repletion". In the accompanies abstract, we have described our recent discovery in that there was a stockpiling of osteoblastic lineage cells during the calcium depletion phase, suggesting that the bone repletion process is initiated during depletion. In this study, we sought to determine the mechanism of this large increase in osteoblastic cells during calcium depletion. We fed mice a calcium-deficient diet for 14 days to initiate the calcium depletion phase, which was followed with feeding mice a calcium-sufficient diet to initiate the calcium repletion phase. With FACS analysis, we found that two MSC subsets of CD45- cells (CD105+ and Sox9+ cells) were upregulated during depletion. With Ki67 expression to assess cell proliferative activity, we further found significant increases in CD45-CD105+CD29+Ki67+ and CD45-CD105+Osx+Ki67+ subpopulations. These findings along with our histologic analysis indicate that there was a marked increase in MSC/osteoprogenitor proliferation and their osteoblastic differentiation during depletion. To further explore MSC/osteoprogenitor cell proliferation and differentiation, mice were injected with BrdU in the middle of the calcium depletion phase. We then isolated osteoblasts after 7 days of dietary calcium repletion. There were significant increases in the individual populations of BrdU+ osteoblastic fraction determined by FACS analysis and in % BrdU+ osteocytes determined by immunohistochemistry. To determine whether this MSC/osteoprogenitor expansion and differentiation also occurred during repletion, we injected mice with BrdU in the beginning of repletion and examined for BrdU incorporation at the end of calcium repletion. We found no increase in BrdU incorporation in subsets of osteogenic cells. In conclusion, bone repletion mechanism is initiated during depletion by ****Truncated - Over Word Limit****

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Other

Imatinib Decreases Lesion Volume, Without Altering Perilesional MRI Tissue Characteristics at 24 Hours post Traumatic Brain Injury

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Traumatic brain injury (TBI) can lead to devastating neurological outcomes with altered vascular networks. However, our understanding of the TBI lesion characteristics remains incomplete. Studies have shown imatinib, also known as Gleevec, can be used as a treatment for cancer (glioblastoma) and stroke. We investigated the effects of Gleevec in a TBI rodent model to determine if it could alter lesion characteristics by modifying the PDGFRÎ± receptor on cells of the neurovascular unit (blood vessels, astrocytes etc) and improve lesion outcomes. We set out to investigate whether non-invasive imaging techniques, such as magnetic resonance imaging (MRI) could detect perilesional changes after treatment with Gleevec. Four groups of adult male Sprague Dawley rats were investigated: 1) sham + Gleevec (SG); 2) sham + vehicle (SV); 3) TBI + Gleevec (TG); 4) TBI + vehicle (TV). TBI was induced using controlled cortical impact (CCI) under stereotactic guidance to the right cortex at a 2.5mm depth. Gleevec (60mg/kg) was administered by injection 1 hour after injury and 24 hours post-injury animals were sacrificed. Ex vivo brains then underwent magnetic resonance imaging at 11.7T. We utilized T2-weighted imaging to identify and visualize lesion characteristics including hematoma and edema. Manual and semi-automatic analysis was performed on T2 scans to extract lesion volumes. Quantitative T2 values for medial perilesion, lateral perilesion, contralesion, contralateral and ipsilateral striatum, contralateral and ipsilateral cortex were extracted. Analysis of T2 images showed 4.2% total lesion volume in the TG group, and 5.83% in TV. We see a significant decrease in total lesion and edema volume in the TG group compared to TV ($p < .05$). It should be noted that no statistical differences appeared in perilesional T2 values. Our findings support the hypothesis that this drug can be used effectively to inhibit activation of PDGFRÎ± receptors expressed in cerebral tissues.

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Faculty

Comparative Effect of Repeated Doses of Oral Glucose vs. Sucrose for Procedural Pain on Urine Markers of Oxidative Stress in Preterm Neonates

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We previously published that a single dose of oral sucrose significantly increased plasma markers of adenosine triphosphate (ATP) utilization (hypoxanthine) and oxidative stress (xanthine, allantoin) in neonates undergoing a clinically required heel lance. However, the effect of repeated doses of sucrose and other sweet solutions such as glucose on above markers is unknown. Using a prospective randomized double blind clinical trial, we measured urinary markers of ATP utilization and oxidative stress in preterm neonates over days of life 3-7. Subjects were randomly assigned to receive either 24% oral sucrose (n=14) or 30% oral glucose (n=13) two minutes before any tissue-damaging procedures (TDPs). Results are compared to control subjects who received standard of care (n=12). TDPs occurred at an average Â± SD of 8 Â± 2 and study drug was administered at an average Â± SD of 7 Â± 6 times over the study period. We found that neonates who received 24% oral sucrose tend to have higher urinary concentration of xanthine compared to those who received 30% oral glucose, specifically at day of life 4. However, the highest urinary concentrations of xanthine (P=0.019) and uric acid (P=0.028) were found in control subjects who received the least amount of oral sucrose analgesia. These findings support our previous findings that untreated pain results in increased ATP utilization and oxidative stress. In addition, this finding suggests that 30% oral glucose may be an acceptable and metabolically less demanding alternative to oral sucrose as a non-pharmacological intervention to procedural pain. Further studies are required to examine more effective ways to decrease procedural pain in preterm neonates.

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Staff

The Association of Kangaroo Mother Care and Energy Conservation in Preterm Infants

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Mentee/Mentor

Objectives: To examine the effect of kangaroo mother care (KMC) on energy utilization as evidenced by reduced biochemical markers of adenosine triphosphate (ATP) degradation, (hypoxanthine (Hx), xanthine (Xa), and uric acid (UA) and oxidative stress (allantoin).

Background: Premature infants admitted to the NICU are at a high risk of suffering the consequences of early maternal separation due to their physiological and metabolic immaturity. Direct effects of KMC are improved mortality and morbidity by stabilization of breathing, oxygen saturation, heart rate, improved breast-feeding and better parent bonding. This is the first study that will link biochemical data to the theorized physiological effects of KMC on the infant's growth and development.

Methods: A quasi-experimental study at Loma Linda Children's Hospital NICU, a tertiary care unit, with an average daily census of 84 babies. Potential subjects are premature infants 28-36 weeks gestation who are medically stable as determined by a SNAPPE-II <9. PI will collect urine at 3 different intervals - Time 0-before KMC, Time 1- after KMC and Time 2-3 hours post KMC. Urine concentrations of Hx, Xa, and UA will be measured using high performance liquid chromatography and allantoin will be quantified using mass spectrometry.

Statistical Approach: Descriptive analysis of data will be examined for assumptions of normalcy. To examine the research question, a repeated-measures analysis of covariance (ANCOVA) will be conducted to assess if mean differences exist on purine levels (energy expenditure/conservation) by time before KMC after controlling for demographic characteristics. The covariates are chosen because of their known effects on energy/expenditure/conservation and may alter purine levels.

Implications for Nursing: This study will supply the physiological data to further support the benefits of energy conservation for recovery and growth in neonates and supply further incentive for all NICU nurses to adopt KMC as the primary modality for caring for premies.

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Staff

A novel patient-derived xenograft model for evaluating therapies that target the CRLF2 signaling pathway to reduce health disparities for Hispanic children with leukemia

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Hispanic children are more likely to develop acute lymphoblastic leukemia (ALL), and when they do, they are more likely to die than other children. A major contributor to this health disparity is a type of high-risk B-cell ALL (CRLF2 B-ALL) that occurs 5 times more often in Hispanic children than others. This leukemia is caused by genetic alterations that result in overexpression of the cytokine receptor, CRLF2. The CRLF2 receptor is activated by the cytokine, TSLP, causing downstream activation of the JAK/STAT5 and PI3/AKT/MTOR pathways. Through distinct mechanisms, both of these pathways are known to upregulate expression of Mcl-1, a Bcl2 family pro-survival molecule. We hypothesized that circulating TSLP present in patients could induce CRLF2 activation leading to increased Mcl-1 expression in CRLF2 B-ALL cells and that inhibiting Mcl-1 might be an effective treatment for this disease. When CRLF2 B-ALL cells from Hispanic pediatric patients were cultured for 3 days with and without TSLP, flow cytometry showed downstream pathway activation and increased Mcl-1 protein expression in cultures with TSLP. CRLF2 B-ALL cells treated in vitro with Mcl-1 inhibitor showed dose-dependent increases in apoptosis, even when physiological TSLP was present. Our next step was to develop a preclinical patient derived xenograft (PDX) model with physiological TSLP for testing the efficacy of Mcl-1 inhibitors against CRLF2 B-ALL from Hispanic patients. We engineered PDX to express physiological levels of TSLP and showed that gene expression profiles in human leukemia cells from these mice, as compared to PDX without TSLP, are more similar to the original patient sample. The PDX mice described here provide a novel in vivo preclinical model for evaluating efficacy of drugs, such as Mcl-1 inhibitor, in context of the background genetic landscape and physiological human TSLP present in patients.

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Other

Physiological Changes in the Stress Circuitry in Response to Western High-fat Diet Consumption

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The hippocampus, amygdala, and prefrontal cortex of the mammalian brain are important in regulating the stress response after psychological trauma. We recently investigated the effects of an obesogenic Western-like high-fat diet (WD) on post-traumatic stress and found that the consumption of a WD during adolescence led to the structural impairment of the ventral and left hippocampus (decreased volumes) and increased volume of the lateral ventricle. These impairments were associated with increased levels of a satiety hormone, leptin, a posttraumatic stress biomarker protein, FKBP5, and also altered behavioral measures of anxiety (Kalyan-Masih et al 2016, revision under review). In order to investigate the alterations to this stress neurocircuitry further, we used quantitative magnetic resonance imaging (MRI) accompanied with immunohistochemistry for astrocytic and microglial responses. We found that diet alone resulted in a fundamental change within tissues of the hippocampus, amygdala, and prefrontal cortex. Using T2 relaxation (ms) readouts we observed decreased T2 values in WD that were not exposed to stress and also in rodents with a control diet (CD) but whom experienced post- traumatic stress. Furthermore, we were able to demonstrate a correlation between amygdala area and decreased T2 values. Additional staining for microglia and astrocytes could reveal changes in cellular structure in response to stress and diet. The significant difference we observed in T2 values could indicate competing effects between diet and stress. Our approach will ultimately allow us to investigate if there is a global change in the functional link between these brain structures associated with stress and speculate about the role of diet on this link. In exploring the underpinnings of this circuitry, we hope to shed light on how the consumption of a WD affects the neural regulators of stress and what preventative measures could be implemented to potentially rescue this circuitry.

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Other

A Rapid Semi-Automated Cell Counting Methodology for Oligodendrocyte Markers

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Traumatic brain injuries (TBI) and other neurodegenerative diseases are known to result in acute and chronic alterations in brain white matter, both in patients and rodent models. In TBI models, having the ability to undertake quantification of oligodendrocytes is an important step in analysis of white matter injury and repair. Presently, there are no established protocols for quantifying oligodendrocytes and their constituent phenotypes. Manual counting can be tedious, time consuming, and subject to bias. We have developed a novel semi-automated cell counting method using Image Pro Premier, for rapid, accurate, and unbiased quantification. We tested our novel method on tissues stained for Olig2 (Oligodendrocyte Lineage Transcription Factor 2) and developed our method to automatically count the number of Olig2 positive cells that colocalized with DAPI (nuclear marker) in the splenium of the corpus callosum. The immunohistochemical slides were captured on a fluorescent microscope as black and white images (16-bit) that were then pseudocolored and further enhanced by adjusting the brightness, contrast, and gamma across all images. Additional criteria were then applied including cell size threshold and colocalization threshold values. An automated cell count was derived using the bright automated cell counts feature. Images were then reviewed for clusters of cells which were computationally separated using the built-in watershed function. Final cell counts were obtained using manual adjustments for splitting and merging of cell errors as a result of the watershed function. Each microscopic image was checked for the deletion of false positives to ensure accurate cell counts. We also undertook manual cell counts to validate our automated method and found no significant differences between manual and automatic counting methods. Thus, we have developed an unbiased semi-automated protocol to rapidly count oligodendrocyte precursors in the white matter of mice. Such protocols will be able to facilitate investigations into neurodegenerative disorders that involve white matter.

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Faculty

Role of NADPH oxidase as a mediator of oxidative damage to low-dose radiation and hindlimb unloading

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Basic Sciences, School of Medicine

The purpose of this study was to determine whether NADPH oxidase-derived oxidative stress can account for unloading and radiation-induced deleterious effects on endothelial damage and neurovascular remodeling in a Nox2 knock-out (KO) mice model. Wild-type (Nox2 (+/+)) C57BL/6 mice or Nox2(-/-) (B6. 129S6-CYBBM) mice placed into one of the following groups (n=5-6/group): age-matched control, hindlimb unloading (HLU), low-dose/low-dose-rate radiation (LDR), or HLU+LDR simultaneously for 21 days, and were then sacrificed at day 7 or 1 month. Anti-orthostatic tail suspension was used to model the unloading, fluid shift, and physiological stress aspects of microgravity. The LDR was delivered using 57Co plates (0.04Gy at 0.01cGy/h) to the whole body in order to simulate the radiation experienced while in microgravity. Brains were isolated for characterization of various oxidative stress markers and vascular topology. Levels of 4-hydroxynonenal (4-HNE) protein, a specific marker for lipid peroxidation, were measured. Expression of aquaporin-4 (AQ4), a water channel protein expressed in astrocyte endfeet, was quantified. Long-term behavioral effects were also evaluated following chronic exposure of radiation + unloading. Thirty days after simulated spaceflight, KO mice showed decreased apoptosis ($p<0.05$) in the brain cortex compared to WT counterparts. The HLU-dependent increase in apoptosis in WT mice was not observed in KO mice. Level of 4-HNE protein was significantly elevated in the hippocampus after hindlimb unloading + radiation compared to controls in the WT mice ($p<0.05$), However, there was no significant differences between treated groups and control in the Nox2-KO mice at 7-day and 1-month time points. In contrast to findings in WT animals, superoxide dismutase level and expression of APQ4 was similar among all KO groups. For behavioral tests, KO mice that received irradiation spent significantly less time in the dark portion of the elevated zero maze than KO controls, suggesting abnormal exploratory/risk-taking behaviors ($p<0.05$).

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Postdoctoral Scholar

Cerebrovasculature after a TBI: are males and females the same?

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Traumatic brain injuries (TBI) occur in 1.7 million people each year in the USA. Little is known about how the cerebrovasculature is altered after TBI. Does a modified brain vasculature lead to functional deficits? We previously reported that TBI elicits acute decrements in cerebral vessels near the injury site in rats. The loss of vasculature is then followed by revascularization over the subsequent 2 weeks.

Sexual dimorphism of the brain is well documented and different hormonal levels in males and females differentially modify the recovery process after brain injury. However, little is known about the effect of gender on the revascularization after a TBI.

Using a model of controlled cortical impact in males and females mice, we set out to determine if the injury and the repair process are different between genders. TBI lesion volume was assessed using MRI T2-weighted imaging at 1 and 8 days post-injury. To evaluate the vascular network, we used a new technique called “vessel painting” that consists of using a fluorescent dye (DiI) to label lipid membranes, thereby staining blood vessels. The brains were imaged using a fluorescence microscope, and vascular parameters such as vessel numbers and complexity were analyzed using Angiotool and Fraclac ImageJ plugins.

We found no differences in lesion volumes between genders. However, revascularization was different. In males, we observed an 18% increase in the number of vessels at the injury site compared to females. This increase translated to a 3.75% enhancement in vessel complexity in the males. We conclude that the damaged cerebrovasculature is repaired more rapidly in males than in females.

Our results imply that the cerebrovasculature recovers differently after TBI, suggesting gender should be considered when patients are being treated for a TBI. Further studies are needed to determine if this difference in revascularization is beneficial or deleterious.

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Postdoctoral Scholar

Comparative Studies of TSLP and IL-7 in Normal Early Human B Lymphopoiesis

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Signals mediated through the IL-7Ra chain, by the binding of IL-7 or TSLP, are known to modulate B cell development in mice. Their roles in human B lymphopoiesis remain controversial. In an earlier work, we demonstrated that with increasing age, IL-7 is as vital for human B cell development as it is for the mouse. In this present work, we investigated a role for TSLP in normal human B lymphopoiesis. We found that bone marrow stromal cells produce TSLP. So we hypothesized that like IL-7, TSLP promoted the proliferation of human B cell precursors but targeted different developmental B cell subsets. To test this hypothesis, we utilized innovative in vitro and xenograft models to evaluate human B cell production in the presence of human TSLP (hTSLP) and IL-7 individually and in combination. First, our in vitro results demonstrate that in the absence of IL-7, TSLP supports the production and proliferation of human CD19+ pro-B cells. Second, our selective cytokine xenografts show that with reduced IL-7, hTSLP increases pro-B and pre-B cells in the bone marrow. Furthermore in vivo, hTSLP in combination with IL-7 increases and maintains the numbers of B cell precursors at each stage of differentiation, compared to IL-7 alone. Collectively, these data demonstrate a function for TSLP and IL-7 signals in human B cell development.

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Faculty

Catalytic Isomerization/Degradation of Methyl-Parathion by Mixed Metal Oxide Nano-Fibers

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Persistent organophosphorus compounds (OPCs) comprise a class of environmental pollutants found in pesticides, that can make their way into surface water supplies. The objectives of this work is to formulate a mechanistic surface mediated models of OPC degradation using LC-MS and computational model data. Development of an efficient catalytic system for OPCs degradation still requires improvements in theory, synthesis, and environmental testing. The results demonstrate: 1) an atomistic mechanistic understanding of organophosphorus degradation chemistry and the role of the catalyst surface chemistries involvement in complete hydrolysis. The atomistic model developed for the model pesticide may be applied to other OPC degradation schemes. Moreover, this research should provide a pathway for large-scale, cost effective remediation pesticides in surface water in agricultural settings.

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Faculty

Silver used to inhibit microbial growth in reusable grocery bags

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LLU SOM and SPH

Reusable grocery bags are seldom washed in homes and can become contaminated with enteric bacteria during use for grocery shopping. The goal of this study was to evaluate the efficacy of a cloth fiber grocery bag impregnated with silver on the control of enteric bacteria in a lab setting and a field setting. The reusable grocery bags were first evaluated for bacterial survival in the laboratory and when used by volunteers over 4 months. In the laboratory, the commercially produced bags were found to reduce Escherichia coli by more than 99.9% within two hours. An additional canvas material was soaked in Dopamine at pH 8, rinsed and soaked again in our laboratories formulation of a bio metallic Ag/Au composite. The canvas swatches produced similar results to the commercially produced reusable grocery bag material. The Commercially produced reusable silver-containing grocery bags were distributed to 38 households in Southern California for use over 4 months and found to have significantly less coliform than non-treated canvas bags. The major significance of these findings is that the broad antimicrobial properties of silver make it a useful component of commercially available reusable grocery bags.

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Staff

Responses of murine microvascular endothelial cells to low doses of gamma rays and charged particles

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A space radiation-associated health risk for astronauts is the potential dysfunction of the cardiovascular system including the microvasculature. We characterized the radiation response of cultured primary mouse heart and retina microvessel endothelial cells exposed to doses of 0 - 50 cGy of 60Co gamma rays, 150 MeV/n protons, 600 MeV/n 16O ions and 600 MeV/n 28Si ions and quantified a series of biomarkers at 24 and 48 hours post-irradiation. Using immunohistochemical methods we observed alterations endothelial specific growth factor receptor VEGFR2/flk-l, which regulates growth and differentiation, nitric oxide synthetase (eNOS/p-eNOS) which regulates of vascular tone, cell junction/barrier function components (ZO-l, VE-cadherin), adhesion proteins (ICAM-l and PECAM) which regulate interactions with circulating leukocytes, thrombomodulin which regulates the coagulation system, oxidative stress (dihydroethidine), and uptake of acetylated low density lipoprotein (Ac-LDL) representing endothelial transport and a link to oxidized LDL metabolism involved in atherosclerosis.

We observed that the various phenotypic markers exhibited cell type, dose, and radiation species-specific expression patterns with gamma rays and oxygen ions including complex dose responses with sensitivity to the lowest dose of 10 cGy. Proton and silicon ion results are currently being analyzed. We also evaluated the coordinated phenotypes of the cells by their ability to differentiate into endothelial tubule networks when grown on a Matrigel + collagen matrix; this represents an early stage of vasculogenesis. Tubule network topological parameters (e.g. tube length, branching) were quantified and found to be modified in the irradiated cells after ~25 cGy of oxygen such that overall network complexity was reduced. Comparisons with other radiation species are ongoing.

Taken together, our studies indicate that charged particles are capable of modifying multiple functional phenotypes of microvascular endothelial cells in heart and retina at doses comparable to those expected during space missions.

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Faculty

Targeting Non-classical Myelin Epitopes to Treat Experimental Autoimmune Encephalomyelitis

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Qa-1 epitopes, the peptides that bind to non-classical major histocompatibility complex Ib Qa-1 molecules and are recognized by Qa-1-restricted CD8+ regulatory T (Treg) cells, have been identified in pathogenic autoimmune cells that attack myelin sheath in experimental autoimmune encephalomyelitis (EAE, an animal model for multiple sclerosis [MS]). Additionally, immunization with such epitopes ameliorates the EAE. However, identification of such epitopes requires knowledge of the pathogenic autoimmune cells which are largely unknown in MS patients. Hence, we asked whether the CD8+ Treg cells could directly target the myelin sheath to ameliorate EAE. To address this question, we analyzed Qa-1 epitopes in myelin oligodendrocyte glycoprotein (MOG that is a protein in myelin sheath). Here, we report identification of a MOG-specific Qa-1 epitope. Immunization with this epitope suppressed ongoing EAE, which was abrogated by CD8+ T cell depletion. Additionally, the epitope immunization activated the epitope-specific CD8+ T cells which specifically accumulated in the CNS-draining cervical lymph nodes. Finally, CD8+ T cells primed by the epitope immunization transferred EAE suppression. Hence, this study reveals a novel regulatory mechanism mediated by the CD8+ Treg cells. We propose that immunization with myelin-specific HLA-E epitopes (human homologues of Qa-1 epitopes) is a promising therapy for MS.

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Other

The Effect Of Tslp On The Expression Of Socs1 And Socs3 In High Risk B-All

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Although most pediatric B-cell acute lymphoblastic leukemias (B-ALL) are highly responsive to standard chemotherapy, the high-risk subset that do not respond well still account for the most childhood cancer deaths. Hispanic/Latino children are more likely to develop high-risk B-ALL than non-Hispanics and when they do they are 39% more likely to die. We know that some of this chemoresistance is due to pathogenic mutations. One such mutation increases the expression of cytokine receptor-like factor 2 (CRLF2) in leukemia cells and it is known as CRLF2 B-ALL. Binding of this receptor by its ligand, TSLP, activates the JAK/STAT5 signaling pathways which leads to the expression of genes promoting cell survival and proliferation. SOCS (suppressor of cytokine signaling) is a family of regulatory proteins that inhibit the TSLP-induced JAK/STAT5 pathways, thereby limiting its changes in gene expression. It is unknown whether TSLP-induced activation of CRLF2 B-ALL cells induces SOCS expression. Our studies examined whether TSLP increases SOCS1 and SOCS3 protein expression in CRLF2 B-ALL cells and if so, whether this change is dynamic. This will help us understand SOCS's potential to inhibit the survival and proliferation of CRLF2 B-ALL cells. We examined the expression of SOCS1 and SOCS3 proteins in vitro using two CRLF2 B-ALL cell lines (MUTZ-5 and CALL-4) cultured with and without of TSLP. SOCS protein expression was evaluated over time (2-7days) using flow cytometry. We found that TSLP-induced increases in SOCS1 and SOCS3 expression in both CRLF2 B-ALL cell lines, which indicates that these leukemia cells have mechanisms to overcome the SOCS effects; these mechanisms will help our understanding of the cancer's in vivo physiological environment. Studying the role of SOCS proteins in high-risk B-ALL oncogenesis will help us target these cancer cells and ultimately, the health disparity affecting Hispanic/Latino children.

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Faculty

Low doses of proton and oxygen particle radiation impact long-term depression in the rat hippocampus and medial prefrontal cortex

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Space radiation consists of protons and nuclei of heavier elements, which present significant risks for astronauts' central nervous systems (CNS). In this study, we used mature male Wistar rats to measure the functional (electrophysiological) effects of very low doses of p+ radiation (0.1, 0.5 Gy; 150 MeV) and 16O (0.01, 0.015 Gy; 400 MeV/n) radiation on synaptic transmission and plasticity in vitro, using slices prepared from the medial prefrontal cortex (mPfc) and the hippocampus (Hpc). A combination of extracellular and intracellular (whole-cell patch-clamp) recordings was used to monitor radiation-induced decrements of synaptic excitability (input-output functions) in in the mPfc layer 2-3 and hippocampal CA3-CA1 pathway. Whole-cell recordings in both regions were used to test passive and active (e.g. action potential) membrane properties and the miniature postsynaptic currents (mEPSCs) reflecting AMPA receptor-mediated excitatory synaptic activity in individual neurons. One month after irradiation with p+ (0.5 Gy) we observed enhanced magnitudes of long-term depression (LTD), a form of synaptic plasticity implicated in reversal learning. Membrane properties of CA1 neurons and mEPSCs were unaffected in the Hpc. In the p+-irradiated mPfc, we only detected subtle increases in synaptic efficacy at 0.5 Gy, while changes of all other endpoints were statistically not significant. In a small cohort of rats irradiated with 16O, our measurements in pyramidal neurons of the mPfc at ~4 months post-irradiation identified radiation-induced decrements in LTD at 0.015 Gy (but not at 0.01 Gy). In addition, at this dose we observed reduced amplitudes of mEPSCs indicating impaired postsynaptic mechanisms of AMPA receptor-mediated signaling. These preliminary results suggest that synaptic networks in the rat mPfc are extremely sensitive to radiation damage, and we are proceeding to test this brain region thoroughly with multiple ions.

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Postdoctoral Scholar

A Biphasic Role of the Active Vitamin D Metabolite in Fracture Repair

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Approximately 6.3 million individuals have to confront fractures annually in the United States. Despite the improvement of clinical management in the past, about 5 ~ 10% fractures proceed to nonunion, not including delayed healing. Patients with nonunion or delayed healing have a lower quality of life and importantly cannot return to normal job duties. In this regard, it is well known that vitamin D is necessary for maintaining the overall bone health through calcium homeostasis. Importantly, several in vitro and in vivo reports suggest a role of vitamin D in enhancing osteogenic differentiation of mesenchymal stem cells and bone strength. These data indicate that vitamin D can potentially be used to promote union and accelerate healing of a fracture. However, recent data have also clearly demonstrated that vitamin D is an anti-inflammatory factor. In the light of evidence that early inflammation is critical for fracture repair, it is crucial to understand how vitamin D may affect initial inflammation stage as well as the fracture healing. Accordingly, we investigated the effects of local administration of 1, 25(OH)2D, i.e. the active vitamin D metabolite, at the early inflammatory and the late non-inflammatory stage on a closed femur fracture. Our data showed that local administration of the 1, 25(OH)2D at early inflammatory stage impaired while at late non-inflammatory stages promoted the fracture healing. We then studied the effects of the early 1,25(OH)2D administration on the cellular recruitment and on the secretion of inflammatory cytokines. Our data showed that local 1,25(OH)2D administration at early inflammatory stage significantly 1) decreased the recruitment of myeloid cells, i.e. the CD11b+, F4/80+, Gr-1+, and CD169+ cells which have been shown critical for fracture repair; 2) suppressed the expression of inflammatory cytokines, i.e. the IL-1α, IL-1β, and TNF-α; 3) inhibited the expression of cyclooxygenase 2 (COX-2) that has been ****Truncated - Over Word Limit****

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Faculty

Impact of Simulated Microgravity on Primary Antibody Response

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The spaceflight environment is known to alter overall immune function, in general, and the distribution many of the cell populations involved in antibody response, specifically. Cytosinephosphorothioate-guanine oligodeoxynucleotide (CpG) is an adjuvant that function by stimulating toll-like receptor 9 (TLR9) to activate and expand innate immune populations.

The purpose of this study was to measure changes in circulating markers of B cell function and stress using hindlimb unloading (HLU) as a ground-based model for spaceflight. We hypothesize that exposure to a spaceflight-like environment will alter immune function, leading to decrements in the ability to generate antibodies in response to an in vivo challenge. We also hypothesize that activating TLR9 signaling will reverse the negative impacts of spaceflight on the antibody response.

Ten week-old female C57BL/6 mice were hindlimb unloaded for a total of 4 weeks. Two weeks after the start of unloading, mice were immunized with either saline, 5 Lf/ml Tetanus Toxoid (TT), 0.4 mg/ml CpG adjuvant, or a combination of TT+CpG. After inoculation, mice were unloaded for an additional two weeks. Mice were euthanized and blood was collected via cardiac puncture. Serum analysis included antigen-specific IgG, Immunoglobulin isotyping, cytokine expression, and corticosterone quantification.

The circulating levels of TT-specific IgG were not impacted by HLU. However, there was an increase in TT-specific IgG levels in mice inoculated with TT+CpG compared to TT only mice, leading to a main effect of CpG ($P<0.05$). HLU did increase circulating corticosterone levels, as well as proinflammatory cytokines.

These results suggest that while simulated microgravity does elicit a stress response, HLU does not appear to change antigen-specific IgG concentrations, nor does unloading impact the efficacy of CpG as an adjuvant. However, this does not preclude the possibility that unloading alters the IgG idiotype distribution. This latter possibility is currently being assessed using NextGen RNAseq techniques.

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Faculty

Immunotherapy for Superficial Bladder Cancer

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Bladder cancer is the most expensive cancer to treat due to high recurrence rates after initial treatment success. New treatments for this disease are therefore needed. The goal of this project is to translate a promising immunotherapeutic for bladder cancer from laboratory to clinic. This immunotherapeutic consists of chitosan (CS) co-formulated with interleukin-12 (IL-12) (CS+IL-12). Completion of the proposed research will enable a Phase I clinical trial for treatment of superficial bladder cancer with CS+IL-12 to commence at the NIH Clinical Center. Endotoxin levels in CS that exceed FDA allowable levels and loss of or negative alterations to the biological properties of CS after sterilization/depyrogenation have hindered clinical translation of CS+IL-12. Our novel technology for depyrogenating chitosan consists of a patented non-thermal nitrogen plasma technique. Proof-of-concept studies have been completed and demonstrate production of sterile depyrogenated CS with retained biological functionality after plasma decontamination. Commercially-available plasma instruments are poorly designed for the treatment of powders and medical applications. This inefficiency necessitates prolonged treatment times and produces excessive heating leading to alterations in CS's physicochemical properties, which dictate its biological properties. Improved plasma instruments optimized for CS decontamination are needed that could subsequently be used to decontaminate other novel materials. We have designed a novel atmospheric pressure non-thermal plasma instrument optimized for plasma decontaminating CS for use in pharmaceuticals and medical devices. The aims of our project include (1) constructing, characterizing, and optimizing the novel plasma instrument, (2) developing and validating GMP protocols for manufacturing and packaging the resultant plasma-sterilized and depyrogenated CS, and (3) to characterize effects of this CS on immune cells in vitro and in vivo. Studies will be incorporated in a new drug application to the FDA to allow clinical testing of CS+IL-12.

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Staff

Two-photon laser scanning microscopy to visualize neurovascular networks and microglia

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Ionizing radiation has been shown to increase macrophage activation post-irradiation. However, little is known about acute microglial activation after low-dose radiation. Multiphoton microscopy is a powerful tool that is especially useful for imaging vascular structure and cellular activity in three-dimensional networks with single-cellular resolution. It is the ideal imaging tool to capture images deep in the brain tissue with very little scattering effect. The aim of this presentation is to identify associations between vessel network and microglia in live brain tissue using two-photon laser scanning microscopy following ionizing irradiation.

In this pilot study, we used a transgenic “MacGreen” mouse model [B6N.Cg-Tg(Csf1r-EGFP)1Hume/J] which expresses Green Fluorescence Protein (GFP) linked to Colony Stimulating Factor 1 Receptor (CSF1R), specific for macrophages and microglia. Two-photon laser scanning microscopy (2-PLSM) was used to track vessel networks and changes in microglial activation in the brain after radiation.

We irradiated eight-month old MacGreen mice with a single, whole-body dose of 3 Gy using a 60Cobalt source. After irradiation, mice were euthanized up to 48 hours post-irradiation. Prior to euthanasia, mice were anesthetized with 3% isoflurane and perfused with 200 Âµl Texas Red-conjugated lycopersicon esculentum (tomato) lectin via the inferior vena cava to label vascular networks. The brain was removed and placed into artificial cerebrospinal fluid to extend tissue viability for ex vivo imaging. Images were collected within 10-60 minutes of euthanasia using an Axio Observer Z1 inverted LSM 710 NLO (Zeiss) coupled to a Chameleon Vision II (Coherent) Ti:sapphire laser (680-1080 nm) emitting at 810 nm to excite the GFP and Texas Red fluorophores simultaneously. Our study suggests that this laser-scanning technique enables direct observation of neurovascular networks and cell population dynamics in live brain.

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Faculty

Filifactor Alocis - Neutrophil Activating Protein-A (Napa) In Virulence And Host Modulation

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Staff

Generation and Genome Editing of Integration-free Induced Pluripotent Stem Cells from Human Peripheral Blood Mononuclear Cells

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Generation of integration-free induced pluripotent stem cells (iPSCs) from adult human peripheral blood (PB) holds great promise for disease modeling and regenerative therapies. We previously reported the use of oriP/EBNA-based episomal vectors (EV) to generate integration-free iPSCs from peripheral blood mononuclear cells (PBMCs). EV is a non-integrating plasmid, which will be gradually depleted from cells after multiple passages. To increase the reprogramming efficiency, we further optimized this system, , including the use of erythroid medium to expand PBMCs before nucleofection, the use of five reprogramming factors KLF4, MYC, BCL-XL, or OCT4 and SOX2, and culturing cells in hypoxia. We found that combinatorial use of these strategies leads to a considerable increase in reprogramming efficiency.

Recently, CRISPR/Cas9 technology has emerged as the most powerful precise genome editing technology to edit mammalian genomes. In this system, sgRNA forms a complex with Cas9 and guides endonuclease Cas9 to target the cognate sequence of the sgRNA. After cleavage of double strand genomic DNA at a certain locus, DNA repair by erroneous NHEJ pathway may lead to gene knockout. Alternatively, the introduction of a plasmid donor with homology arms that are identical to DNA surrounding the cleavage site may lead to precise gene knockin at that locus. CRISPR makes it possible to target genes of therapeutic value to a safe locus on the genome, which will not induce adverse effects. After optimizations on sgRNA design and using strong promoter to express Cas9, we achieved an up to 10% knockin efficiency in iPSC lines. We will further identify small molecules and new factors to increase gene knockin efficiency in iPSCs.

In summary, our optimized EV reprogramming system is very efficient in generating integration-free iPSCs from adult peripheral blood. In addition, we are able to precisely edit genome of iPSCs by CRISPR/Cas9. This affordable EV reprogramming system ****Truncated - Over Word Limit****

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Postdoctoral Scholar

A Novel Calcitriol-producing, Gut-homing CD11b+Gr-1+ Macrophage Specifically Homes to the Inflamed gut and Robustly Suppresses an Immune-mediated Experimental Colitis

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Inflammatory bowel disease (IBD) is an incurable chronic disease that affects approximately 1.4 million Americans. Current medical therapies are aimed at suppressing systemic immune responses that are involved in chronic inflammation. One remaining therapeutic challenge is the regeneration of the damaged mucosal barrier in a timely manner, which may preclude a chronic inflammatory milieu and any subsequent fibrosis. We recently reported that intravenous delivery of CD11b+Gr-1+ macrophages engineered for producing the active vitamin D metabolite (i.e. 1,25[OH]2D or calcitriol) effectively improved mucosal repair and local inflammation control in an intestine injury IBD model, i.e. dextran sulfate sodium(DSS)-induced colitis. In this study, we evaluated the therapeutic efficacy of this novel strategy to treat a Th1-mediated colitis, i.e. 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis. Additionally, cellular infiltration and migration of the injected cells (GFP+) were analyzed by H&E staining and immunohistochemistry. Finally, how calcitriol regulates colonic stem cells in situ after inflammation was carefully investigated. Our data suggested that CD11b+Gr-1+ macrophages engineered for expression of a gut-homing receptor (CCR9) and for constitutive production of calcitriol have specific homing capacity towards inflamed intestines, provide targeted calcitriol to the inflamed bowel to effectively suppress the inflammation, and promote epithelial regeneration through regulating local colonic stem cells' survival and migration. Hence, this novel calcitriol-producing, gut-homing CD11b+Gr-1+ macrophages described in this study is a potential effective therapy for IBD patients.

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Postdoctoral Scholar

TGFB's Role in WNT Induced Neural Crest Cells from Human Embryonic Stem Cells

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The embryonic origin of disparate cell types such as melanocytes, neurons, glia, smooth muscle, osteocytes and chondrocytes of vertebrates stems from neural crest cells (NCCs). The signaling pathways and factors involved in the induction of this transient population of cells has been studied heavily in the commonly utilized model organisms, Chicken, Zebrafish and Xenopus. The general consensus suggests that NCCs are induced at or before embryonic gastrulation. Our lab has engineered a fast and efficient model of human neural crest development based on WNT signaling from human embryonic stem cells, ESCs. Here we further characterize the temporal requirements and effects of WNT signaling, and identify the reason for the controversial contribution of TGFβ/Activin in the derivation of NCCs from ESCs as previously reported by us and other groups. Furthermore, we present a novel WNT mediated condition that results in a more robust generation of NCCs, which results in the generation of NCCs not restricted to a cranial population but expanded to include NCCs with caudal identities.

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Postdoctoral Scholar

Mechano-Modulation Of Induced Pluripotent Stem Cells By Electrospun Scaffolds To Enhance Developmental Stage-Specific Differentiation

Maricela Maldonado (1), Rebeccah J. Luu (1), Gerardo Ico (1), Alex Ospina (1), Danielle Myung (1), Hung Ping Shih (2), and Jin Nam (1)

(1) University of California, Riverside; (2) City of Hope

The field of mechanobiology has been widely studied for multiple adult stem cell types to enhance their differentiation efficiency. However, little is known about the effects of the temporally changing cellular microenvironments on the step-wise differentiation of human induced pluripotent stem cells (IPSCs) to end-phenotypes. Traditionally, research has primarily focused on optimizing the biochemical cues to enhance the differentiation of PSCs using various lineage- and stage-specific growth factor/cytokine combinations while the effects of the temporally regulated mechanical microenvironment on the differentiation of PSCs remain elusive. In this study, we examined how electrospun scaffold stiffness affects the differentiation of IPSCs along the developmental stages of differentiation to motor neuron, pancreatic endoderm, or chondrocyte phenotypes. IPSCs were seeded onto collagen type I-conjugated electrospun scaffolds with distinct mechanical properties (Reduced Young's Modulus = 19kPa (Soft) or 313kPa (Stiff)) at each developmental stage and samples were analyzed for lineage stage-specific gene and protein expression using rt-PCR and immunocytochemistry, respectively. We demonstrate that a specific stiffness at each stage (soft or stiff) can enhance the differentiation to all three germ layer derivative cell types. Initial ectodermal differentiation was enhanced on soft scaffolds, but as the differentiation progressed stiff scaffolds enhanced neural progenitor and motor neuron specification. In contrast, mesendodermal differentiation was significantly enhanced on stiff scaffolds but further specification to posterior foregut required a soft scaffold. Interestingly, mesodermal specification required a stiff scaffold. Both pancreatic endoderm and chondrocyte differentiation was enhanced on soft scaffolds. Overall, these results demonstrate the significant role of mechano-modulation to enhance the differentiation efficiency of pluripotent stem cells to distinct cell phenotypes. Further optimization of both the biochemical and mechanical microenvironment could result in a higher differentiation efficiency which can further facilitate translational applications of the cells.

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Faculty

Silver Nanoparticles Exhibit Coating and Dose-Dependent Neurotoxicity in Glutamatergic Neurons Derived from Human Embryonic Stem Cells

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Silver nanoparticles (AgNPs) are used extensively as anti-microbial agents in various products, but little is known about their potential neurotoxic effects. In this study, we used glutamatergic neurons derived from human embryonic stem cells as a cellular model to study 20 nm citrate-coated AgNPs (AgSCs) and Polyvinylpyrrolidone-coated AgNPs (AgSPs) induced neurotoxicity. AgSCs significantly damaged neurite outgrowths; increased the production of reactive oxygen species and Ca2+ influxes; reduced the expression of MAP2, PSD95, vGlut1 and NMDA receptor proteins at concentrations as low as 0.1 Âµg/ml. In contrast, AgSPs exhibited neurotoxicity only at higher concentration. Furthermore, our results showed that AgSCs induced glutamate excitotoxicity by activation of calmodulin and induction of nitric oxide synthase; increased the phosphorylation of glycogen synthase kinase-3 a/Î² at Tyr216 and Tau at Ser396 and reduced the expression of Tau46, which are typically observed in Alzheimer's disease. This study indicated that stem cells can provide an excellent platform for studying nanoparticle induced neurotoxicity.

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Postdoctoral Scholar

The P2X7 Receptor Is an Upstream Regulator of Dynamic Blebbing and a Pluripoteny Marker in Human Embryonic Stem Cells

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Dynamic blebbing occurs during passaging of pluripotent stem cells and inhibits cell attachment and survival. New methods are needed to reduce blebbing during passaging. Our purpose was to test the hypotheses that the P2X7 receptor, which is activated by extracellular ATP during passaging, initiates dynamic blebbing. The P2X7 receptor was found in human embryonic stem cells (hESC) using PCR and immunocytochemistry, but not in differentiating cells. Extracellular ATP concentrations were 14 x higher in medium during passaging. Addition of ATP to culture medium prolonged dynamic blebbing and inhibited attachment. Inhibition of P2X7 by specific drugs or by siRNA greatly reduced dynamic blebbing and improved cell attachment. Because P2X7 is a calcium channel, cells were incubated in calcium chelators (EGTA or BAPTA) and blebbing was reduced and attachment improved. Calcium influx was observed using Fura-4 when ATP was added to culture medium and inhibited in the presence of the P2X7 inhibitor. ROCK inhibitors, which we found inhibit dynamic blebbing, are widely used to help cell attachment during passaging. Because Rac often counteracts the ROCK pathway, we examined activated Rac and Rho in dynamically blebbing and attached cells. Rac activity decreased in dynamic blebbing cells. Over-expressing activated Rac in hESC reduced blebbing and promoted cell attachment. Results were validated with a Rac inhibitor which also prolonged dynamic blebbing and reduced cell attachment. These data identified a pathway involving P2X7 that initiates and prolongs dynamic blebbing during hESC passaging. This pathway provides new insight into factors that increase dynamic blebbing, such as release of ATP from cell that die during passaging and identities new targets, such as P2X7, that could be used to decrease dynamic blebbing and improve cell attachment and survival. These results may lead to better ways to control dynamic blebbing in cultured hESC and further show that hESC are an ****Truncated - Over Word Limit****

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Postdoctoral Scholar

Osteochondral Tissue Morphogenesis of Human Mesenchymal Stem Cells under Dynamic Gradient Strain

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Biomechanical forces have been shown to significantly affect tissue morphogenesis, pathogenesis and repair, especially in orthopaedic tissues. Such biological processes are critically related to functional behaviors of mesenchymal stem cells (MSCs). However, the fundamentals regarding how mechanical forces direct MSC differentiation and subsequent tissue formation are still elusive. We hypothesize that such a mechano-regulation is magnitude-dependent to elicit differential responses from MSCs to form an osteochondral tissue. Core-shell electrospun scaffolds were synthesized to generate a gradient of mechanical properties with respect to the thickness of the scaffold, resulting in a strain gradient under dynamic compression. Human MSCs were cultured within the scaffolds and subjected to daily compression (8.5% at a frequency of 1 Hz for 2 hours/day for up to 28 days). Under the culture condition in osteogenic media, gene and protein expression of osteogenic markers (e.g., COL1A1, SPARC, RUNX2, calcium deposition) had noticeable decreases while chondrogenic markers (ACAN, COL2A1, SOX9, GAG synthesis) were upregulated as the local compressive strain increased. The dynamic modulus of the engineered tissues were proportionally increased as the amount of osteogenic phases (low local strain areas) increased. Overall, we show that the degree of differentiation of hMSCs towards osteogenic or chondrogenic lineage is inversely related and depends on the magnitude of local dynamic compressive strain. These results demonstrate that multi-phenotypic differentiation of hMSCs can be controlled by varying the local strain magnitudes, providing a novel strategy to modulate differentiation specification and osteochondral tissue morphogenesis.

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Student

Influence of hypoxia on the stemness of umbilical cord matrix-derived mesenchymal stem cells cultured on chitosan films.

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Chitosan is attractive as a substrate for stem cell expansion because improves stemness through formation of spheroids. Hypoxia has also been proposed as a strategy to enhance stemness and survival of stem cells after in vivo implantation. This study was therefore designed to evaluate the influence of hypoxia on chitosan-induced behavior of stem cells. Umbilical cord matrix-derived stem cells were cultured on chitosan film or standard plate under normoxia and hypoxia, for 3 and 7 days. Hypoxia generally increased the volume and number of spheroids formed on chitosan, but the cellularity of cultures on chitosan films remained lower than that of standard plates. After 7 days of culture, the expression of stemness related genes (Oct4, Sox2, and Nanog) was best stimulated by combined exposure to chitosan and hypoxia, chitosan stimulating predominantly Oct4 expression while hypoxia upregulated mainly Sox2 and Nanog expression between day 3 and 7. Combining hypoxia to chitosan was more effective than each condition alone at enhancing the expression of stemness genes between day 3 and 7. Based on our results, conditioning stem cells for 7 days on chitosan films under hypoxic conditions is recommended to enhance the stemness of stem cells, and minimize cell loss due to lack of attachment on chitosan.

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Student

Direct culture of bone marrow derived mesenchymal stem cells on 8YSZ

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Biomaterials and Nanomedicine Lab Bioengineering Department, Window to the Brian PIRE NSF

The NSF PIRE Window to the Brain (WttB) Project is an international collaboration between nine research groups: five at UCR, one at UCSD, and three top institutions in Mexico. WttB is a five-year project entering its second year, with the goal of developing a transparent cranial implant, â€œa window,â€ from yttria stabilized zirconia (8YSZ) nano-powder. This material choice stems from the need for a cranial implant exhibiting both toughness and transparency. In the future, such an implant will be used for diagnostics, photodynamic therapies, and real-time imaging of specific biological markers linked to traumatic encephalopathy. Last year, recent achievements were made in 8YSZ sample fabrication. As the collaborating biomaterials and nanomedicine research lab, our goal was to run the first cytocompatibility test on one unpolished sample. Bone marrow mesenchymal stem cells (BMSCs), isolated from rat weanlings, were chosen for their ability to be directed down an osteogenic lineage. The BMSCs response provides us with valuable information pertaining to whether such an environment is conducive to cellular adherence and healthy cellular morphology. The 24-hour study consisted of BMSCs in direct culture on the 8YSZ unpolished sample. Once the 24-hour culture was complete, fluorescent staining was used to assess cellular morphology of BMSCs seeded directly on the 8YSZ sample. In addition, cells in direct culture with the 8YSZ sample, but growing on the plate were also imaged. These images were compared to the control. Future work will include cytocompatibility studies on polished 8YSZ samples. This first study concludes that BMSCs were able to grow on 8YSZ. The images collected gave us valuable preliminary data on BMSC morphological response when grown on unpolished 8YSZ. Such results further the WttB Project and yield information on BMSC responses to YSZ ceramics.

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Student

Cytocompatibility of Porous Magnetic Nanocomposites with BMSCs

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Magnetic nanocomposite is a type of material that composed of more than one phase, one or more phase has a size of less than 100 nm in one, two or three dimensions, and possesses magnetic properties. Poly(glycerol sebacate) (PGS) has been extensively explored for different tissue engineering applications such as myocardial vascular graft, heart valve, contact guidance and drug delivery. Magnetic nanoparticles (MNPs) were reported to be able to control stem cell differentiation, hence, we introduced MNPs into PGS substrate to provide nanofeatures and enable the response of PGS nanocomposites to external magnetic force. This is the first study on magnetic PGS nanocomposites, which was investigated for potential tissue engineering applications. The objectives of this first study were to create porous magnetic PGS nanocomposites with 0, 0.5, 1, 5 and 10 wt% incorporation of MNPs, characterize the structure and study the cytocompatibility of the magnetic nanocomposites with bone marrow-derived mesenchymal stem cells (BMSCs). The study showed that the porous magnetic PGS nanocomposites were cytocompatible with BMSCs and allowed the penetration of BMSCs into the nanocompoaistes. The nanocomposite with 10 wt% of MNPs incorporation was favored by cells for initial attachment when compared with other groups. Surface degradation of the nanocomposites might contribute to the lower cell density on the samples than on the plate. External magnetic fields will be applied in future studies to investigate their influence on biological functions of BMSCs grow on porous magnetic PGS nanocomposites.

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Student

Video Bioinformatics Analysis of Pluripotent Stem Cell Morphology, Quality, and Cellular Dynamics Cultured in Defined Conditions

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StemCellQC is a powerful quantitative video bioinformatics tool for use with label free time-lapse images. It can perform high content profiling and analysis of dynamic processes in living pluripotent stem cells (PSC). It was created out of a foundational need for software tools that can be used to monitor the behavior and quality of PSC in culture. Our objective was to demonstrate the functionality of StemCellQC by comparing the morphology, quality, and dynamic processes of human embryonic stem cell (hESC) colonies grown using defined conditions. In one application of the program, H9, H1, and CC3 PSC were cultured on Matrigel in mTeSR1 for 70 hours in a BioStation CT and analyzed by StemCellQC. H9 hESC exhibited the most desirable colony traits of the three cell lines. H9 hESC were the largest in size and had higher solidity than H1 or CC3 PSC. While H1 and CC3 PSC had an increasing brightness/area ratio with time, the H9 hESC colonies had a consistently low brightness/area ratio signifying less cell death. Motility was significantly less for the H9 hESC suggesting improved colony attachment that facilitated colony growth and health. In a separate experiment, H9 hESC were grown on Matrigel, Geltrex or laminin-521 for 48 hours in a BioStation CT and analyzed by StemCellQC. Overall, laminin-521 performed the best of the three substrates. Attachment on laminin-521 was more efficient than on Matrigel or Geltrex. Colonies grew at about the same rate on the three substrates with laminin-521 supporting slightly faster growth. Laminin-521 exhibited the least amount of cell death at the surface of colonies with a consistently low brightness/area ratio supported by Trypan blue and MitoSox biomarkers. These data provide examples of its potential use of StemCellQC in basic research, translational labs, toxicology and drug testing, and clinical facilities engaged in stem cell therapy.

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Student

A new stem cell model of amyloid-beta toxicity in Alzheimer's Disease

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Cal State San Bernardino, The Beckman Research Institute at City of Hope Medical Center, and The Sidell-Kagan Foundation

The scientific community has been aware of Alzheimer's disease (AD) for over 100 years and began intensive investigation in the late 1970s. Unfortunately, this has not led to any effective treatments or a mechanistic understanding of the underlying disease process. Two of the constant features of AD patient brains is accumulation of amyloid-beta (A-beta) plaques and evidence of an abnormal accumulation of vesicles in neurons that will ultimately die. A-beta plaques are largely made of an aggregated form of A-beta42 peptide generated by proteolysis of amyloid precursor protein (APP). The abnormal vesicles accumulating in AD neurons have proteins identifying them as fusion of autophagy, endosomal, and lysosomal (AEL) organelles. Using TALEN we edited one allele of the App gene in H9 human embryonic cells (hESCs). The edited allele replaced App with a cassette that directly expressed A-beta rather than the full length APP thus eliminating the need for proteolytic processing. An additional feature of our editing was to include the secretory signal peptide ensuring that the A-beta was routed through the same cellular pathway as APP. This created a panel of isogenic cell lines expressing APP, A-beta40, or A-beta42 all under the same regulatory control.

In this study, I present my initial results of phenotypic variation of our isogenic cell lines after differentiation into neurons. The culture phenotypes were measured by quantifying neuronal cluster size and number as the cultures age. Immunological staining was also done to view the distribution and intensity of different neuronal and AEL markers. Preliminary data reveals that among expression of neuronal targets and culture morphology there is no difference between control and edited lines during differentiation. Following differentiation, the A-beta42 lines show differences in morphology as they age.

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Student

Bcl-2 family pro-death protein expression is regulated by the TSLP cytokine in B-cell precursor Leukemia.

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Background. The cytokine, TSLP, stimulates in vitro proliferation of human fetal B cell precursors. Genetic alterations that cause overexpression of the TSLP receptor component, CRLF2, lead to B cell acute lymphoblastic leukemia (CRLF2 B-ALL), implicating the TSLP-CRLF2 pathway in leukemogenesis. When TSLP binds, the receptor initiates downstream JAK2/STAT5 and PI3/AKT/mTOR pathway activation. Activation of these pathways has been associated with chemoresistance and their downstream targets include members of the Bcl2 family. Specifically, our goal was to look at pro-apoptotic Bcl-2 family members and TSLP's treatment effect on this family's expression.

Methods. Human CRLF2 B-ALL cell lines (MUTZ-5 and Call-4) were cultured with and without TSLP and evaluated at different time points by flow cytometry. Viability of cells was also determined using the Fixable Viability Dye eFlour 450 and flow cytometry.

Results. These data show that TSLP prompts an increase in BCL-2 pro-apoptotic molecules Bad, Bak, Bim, and Bax. TSLP was also shown to increase the rate of apoptosis in cells. A reduction in cell numbers was seen for the TSLP treated population when compared to the non-treated population.

Conclusion. These data suggest that under in vitro conditions TSLP activated CRLF2 B-ALL cells are likely to express high levels of pro-apoptotic molecules contributing the death of the leukemia cells. These TSLP treated cells have shown to express a higher concentration of Bcl-2 pro-apoptotic proteins than the non-treated cell lines.

Future Studies. TSLP has also been shown to have an important role in the production of normal B cells. Studies will be conducted evaluating normal B cell growth and BCL-2 family expression with and without TSLP treatment. Also, future studies will be aimed to determine the effect of TSLP on Bcl-2 family protein expression in normal and malignant B cell progenitors in an in vivo patient-derived xenograft mouse model.

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Student

Novel Hydroxyapatite Coatings Reduced Degradation of Magnesium Implants and Promoted Bone Marrow Derived Mesenchymal Stem Cell Adhesion

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Magnesium (Mg) and its alloys have high potential to serve as biodegradable metallic implants for medical applications due to its attractive mechanical and osseointegration properties. Hydroxyapatite (HA) is found in natural bones, which shows excellent biocompatibility, bioactivity, and osteoconductivity. Therefore, HA is a suitable coating material for reducing the degradation rate and improving bioactivity of Mg-based implants. In this study, the nano-size HA (nHA) and micron-size HA (mHA) coatings were deposited on Mg disc samples with a diameter of 8 mm and a thickness of 1 mm using N2 Biomedical's proprietary process. We investigated the degradation of nHA and mHA coated Mg in the revised simulated body fluid (rSBF). We also examined the cytocompatibility of nHA and mHA coated Mg with bone marrow derived mesenchymal stem cells (BMSCs). During the 6-week immersion in rSBF, the mass loss of nHA and mHA coated Mg were significantly less than non-coated Mg. BMSCs attached and exhibited normal morphology on the surface of Mg-based samples after a 24-hour culture. Both nHA and mHA coated Mg showed great cytocompatibility with BMSCs, and mHA coating remained intact after the cell culture. In addition, all HA coated samples showed higher BMSC density when compared to non-coated Mg. In summary, hydroxyapatite coating can significantly reduce the degradation and improve the bioactivity of Mg-based implants. In the future, we will further investigate these samples under mechanical loading and circulating body fluid to closely mimic in vivo conditions.

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Student

Mechano-transduction and human Neural Crest Induction

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Neural crest cells (NCC) are a multipotent population of cells that give rise to various derivatives, including peripheral neurons and glia and craniofacial bone and cartilage. NCC abnormalities lead to neurocristopathies, such as cleft lip and palate. We have recently developed a Wnt based model for human NCC induction from hES which is the fastest and most efficient model reported, generating above 60% NCC induction (PAX7-SOX10 co-expression) in 5 days. Similar to all other models of hNCC induction published, our protocol is performed on glass/plastic slides (~50GPa or ~3GPa respectively), which provide stiffness magnitudes significantly higher than that of various mammalian tissues (~4.75kPa to ~14.8kPa for brain tissue; up to ~40kPa for collagenous bone). Mechanical cues have recently been recognized as important signals that regulate gene expression and fate decisions. However, the precise effect(s) stiffness may have on NCC formation and development are not defined.

To test the effects of substrate stiffness on NCC induction we used polyacrylamide gels (PAAG) and polydimethylsiloxane (PDMS) to create substrates with stiffness ranging from 100Pa to 1.3 MPa. While robust NCC induction is seen on glass/plastic, induction considerably drops at 3 kPa to 1.3Mpa, and no induction was seen at 350Pa to 1kpa. To improve induction under low stiffness we modulated signaling pathways associated with NCC induction (Wnt, BMP and FGF). However, this proved insufficient to compensate for decreased induction.

Pathways of stiffness sensing and response include the ROCK/Myosin II pathway, whose activity is affected by glucose levels. We show that increased ROCK inhibition duration leads to increased NCC induction. Glucose concentration representing pre-diabetic levels in adults (10mM) shows greater NCC induction compared to normal(5mM) and diabetic levels(25mM). Stiffness, glucose levels, and ROCK/Myosin II pathway activation have the potential to alter cytoskeletal stiffness, which affects mechanotransductive cues involved in cell function and behavior.

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Student

Role of Insulin in Early Neural Crest Specification

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Neural Crest (NC) cells arise early in development, migrate extensively, and contribute to various derivatives, and our unpublished work strongly suggests that NC formation is initiated during blastula stages, earlier than previously published data shows. Wnt, BMP, and FGF, among other growth factors, are implicated in NC specification; however, no studies have addressed possible signaling contributions towards NC in animal models at this early stage. Furthermore, a potentially instructive role of Insulin and its intracellular signaling branches in early NC development has not been established, despite its role in NC growth media and implications in literature relating to neurocristopathies. This work presented here examines the significance of the Insulin signaling pathway in formation of NC in chick and our human NC model.

We report that a hypoinsulinemic environment or inhibition of Insulin signaling via receptor inhibition prevents Pax7 expression in chick. Analysis in our hNC model shows that simultaneous inhibition of IR and IGFR reduces expression of NC marker. Furthermore, targeting PI3K and PDK1 also shows a major reduction in Sox10; however, Pax7 is only moderately affected or increased by these inhibitions. Alternatively, targeting MEK shows a major reduction in Pax7 while increasing Sox10. These findings support our hypothesis that Insulin signaling is necessary for early NC specification. Interestingly, inhibition of the PI3K signaling branch supports expression of early NC markers but reduces expression of late markers and inhibition of MAPK signaling cascade improves expression of later NC markers. Altogether, this suggests a requirement of Insulin signaling for NC specification and PI3K signaling for propagation of NC development. This work identifies a novel instructive role of Insulin signaling in early NC development, and may provide insight into the regulatory role of Insulin signaling in neurocristopathies.

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Student

Generation of cortical neurons from human embryonic stem cells for neurotoxicity testing of tobacco products

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We have developed a neurosphere-based stem cell neuronal differentiation protocol, which can recapitulate corticogenesis and produce neuronal cell types that are similar to upper and lower cortical layer including neurons found in the germinal zone of the developing human cortex. In this study, we use this platform to investigate the neurotoxic effects of different tobacco products. Cigarette butts contain over 4000 chemicals many of which are known to have neurotoxic effects. Aqueous cigarette tar extracts (ACTEs) generated from smoked cigarette butts were applied at different concentrations to neuronal progenitors and cortical neurons derived from human embryonic stem cells. Our results demonstrated that, ACTEs reduced the expression of cortical neuronal progenitor markers pax6, tbr2, and neuroD; decreased the number of cortical layers neurons (tbr1, satb2, foxp2, brn2), reduced synaptic proteins PSD95, Synaptophysin and vesicular glutamate transporter1 (vGlut1) expression after exposure to extract from one smoked cigarette butt. Electronic cigarettes are newly emerging tobacco products that are designed to be refillable with nicotine-containing e-liquid. The main ingredients in e-liquid solutions are propylene glycol, glycerin, water, nicotine, and flavorings. The use of e-cigarettes has raised interests on e-liquid toxicology and safety, which focus on exposure and dose response of different e-liquid products. Further studies are needed to compare the neurotoxicity effect of cigarette butt extracts, e-liquid and nicotine. Our stem cell neuronal differentiation protocol is an excellent platform to assess the benefits and risks of using different tobacco products.

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Student

Targeting the Long Form Prolactin Receptor as a Therapeutic Against Cancer Stem Cells

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Cancer stem cells (CSCs) are believed to be responsible for relapse of cancer in patients who were in remission. Prolactin (PRL) and its receptor, particularly the long form prolactin receptor (LFPRLR) are known to be expressed in high amounts in people afflicted by breast and prostate cancer. The LFPRLR is also known to be expressed in cancer cell and stem cell populations. Therefore, prolactin signaling may contribute to disease progression partly through its effects on CSCs. The Walker lab has shown that using a LFPRLR splice modulating oligomer, LFPRLR SMO, knocks down expression of only the LFPRLR. To explore more potential mechanisms on how prolactin signaling regulates CSCs, we utilize the tumorsphere formation assay to isolate CSCs and examine the effects of LFPRLR knockdown on CSCs. The tumorspheres from the human breast cancer cell line, T47D, are reduced in size and number in response to LFPRLR SMO. In addition, other data from the lab show that knockdown of the LFPRLR using the LFPRLR SMO eventually kills CSCs (>95% of CSCs underwent apoptosis when compared to DPBS and control SMO treatment). Furthermore, results from another lab member also shows a reduction in the expression of ABC transporters, ABCA1 and ABCC6, and one of the stem cell markers, cMyc, in PC3 CSCs, a human prostate cancer cell line. These results collectively imply that targeting the LFPRLR could increase sensitivity to chemotherapeutic agents as well as directly reducing cancer relapse.

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Student

A Novel Approach to 3D Tissue Preparation of Cardiac Progenitor Cells using the “Kenzan” method

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Endogenous cardiac progenitor cells (CPC) isolated from the heart represent a promising source of cells for cardiac cell therapy. Isl-1+ C-kit+ CPCs have several markers that characterize them as early cardiovascular progenitors, and have the ability to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells. They secrete paracrine factors, proliferate and differentiate in vivo at the site of infarction after administration by direct injection in an ovine model. We tested the hypothesis that three-dimensional (3D) CPC patches of early, clonal CPCs can be printed using the “Kenzan" method, allowing early cardiovascular progenitors to be administered in customized patches of undifferentiated, but cardiovascular committed progenitor cells for repair of the heart. 3D printing using the “Kenzan” method involves a “Kenzan” needle array, which confers scaffold-free, high-density cell architecture. Patches provide a method for optimizing cell number and delivery at the site of infarction when used for cardiovascular repair. Neonatal CPC clones were grown as 3D cell aggregates (cardiospheres) to a diameter within a range of 500 to 600 um, in order to be utilized by the “Kenzan”. Cardiospheres were then printed using the Regenova Bio 3D printer by Cyfuse. The spheroids were placed onto the “Kenzan” according to a pre-designed 3D shape. The 3D design included a 1 cm x 0.5 cm x 1mm patch. The 3D printed structure was removed from the “Kenzan” and allowed to mature and fuse. The 3D printed structure was then fixed using 4% paraformaldehyde and placed in OCT for immunohistochemistry. The printed patch was examined by immunostaining and was shown to retain an undifferentiated, islet-1 status. Future directions include transplantation post infarction in an ovine model.

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Student

TSLP cytokine effects on cell survival in B-cell precursor leukemia

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Background: The cytokine, TSLP, stimulates in vitro proliferation of human fetal B cell precursors, however, its in vivo role during normal human B lymphopoiesis is unknown. The overexpression of the TSLP receptor component, CRLF2, results in B-cell precursor acute lymphoblastic leukemia (CRLF2 B-ALL), implicating the importance of the TSLP-CRLF2 pathway in leukemogenesis. When TSLP binds, the receptor initiates downstream JAK2/STAT5 and PI3/AKT/mTOR pathway activation. Activation of these pathways is associated with oncogenesis and has been associated with increased cellular proliferation and survival. Protection from apoptosis is an important cellular mechanism in the development of leukemia. TSLP is implicated to lead to changes in the regulation of apoptosis. Activation of enzymes known as caspases is an early event in the process of apoptosis and results in the cleavage of protein substrates and subsequent disassembly of the cell. Activation of caspase-3/7 will provide a method of measuring TSLP-induced apoptosis in CRLF2-B-ALL cell lines.

Methods: Human CRLF2 B-ALL cell lines (MUTZ-5 and CALL-4) were cultured with or without TSLP. Apoptosis in each cell line was evaluated by flow cytometry using assays that measure caspase-3/7 activity by cleaving a peptides substrate.

Results: Our data show that cell survival decreases in both CRLF2 B-ALL cell lines after TSLP treatment. TSLP also increased the percentage of apoptotic cells in both cell lines. MUTZ-5 cells showed up to a 2-fold increase in the percentage of cells with caspase-3/7 activation, while CALL-4 showed up to a 5-fold increase after 3 days in TSLP treatment.

Conclusion: Our data suggest that TSLP stimulates caspase-3/7 activation in CRLF2 B-ALL cells under in vitro conditions. This suggests that TSLP treated leukemia cells are more likely to undergo cell death by apoptosis.

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Student

A novel video bioinformatics toolbox to study mitochondrial morphology, dynamics, and mitophagy in stem cells

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Live cell imaging coupled with video bioinformatics software tools is a powerful technology for studying changes in mitochondrial morphology, dynamics, and health. Video bioinformatics tools were used to assess the effects of electronic cigarette (EC) liquids and aerosols on mitochondria health. Mouse neural stem (mNSC), selected for their well-defined mitochondria, were nucleofected to stably express a MitoTimer reporter with the colorimetric Timer protein tagged to the cytochrome c oxidase subunit VIII gene. MitoTimer-mNSC report on mitochondrial protein oxidation levels by an irreversible fluorescent shift from green to red. Time-lapse images were collected at millisecond resolution, and bioinformatics software was developed to quantify protein oxidation based on the relative red/green fluorescence. To induce stress, MitoTimer-mNSCs were treated for 24 hours with EC liquids or aerosols from a major tobacco company. The liquid/aerosol treated cells showed significant elevation in mitochondrial protein oxidation ($p<0.01$ versus untreated controls). Oxidation was preventable by a reactive oxygen species scavenger, N-acetyl-L-cysteine (NAC). The mitochondria were then segmented using CellProfiler and classified as round, networked, or swollen. EC aerosol/fluid-treated mNSC exhibited a dose-dependent shift from the round (control) to networked (low dose) to swollen (high dose) phenotypes. Mitochondrial dynamics were quantified using a pixel-based motion magnification algorithm written in Matlab. TMRM fluorescence, which measures mitochondrial membrane potential, was lost at high doses, indicating leaky, damaged mitochondria. To determine if EC liquid/aerosol can induce mitophagy, a three-channel mitophagy-reporter system was developed. EC aerosol-treated cells exhibited an increase in the number and size of autophagosomes compared to controls. Video bioinformatics software showed that exposure to EC fluid/aerosol increased oxidation of mitochondrial proteins, disrupted mitochondrial morphology and membrane potential, and increased autophagy. Disruption of normal mitochondrial dynamics and autophagy cycles can cause deleterious effects, which have been linked to neurodegenerative disorders, cancer, and aging.