

2017 CELL BIOLOGY RETREAT

THE DEPARTMENT OF CELL BIOLOGY THE CENTER FOR VASCULAR BIOLOGY THE CELL BIOLOGY GRADUATE PROGRAM

Tuesday, April 18, 2017 8:30am-6:00pm



The Pond House at Elizabeth Park 1555 Asylum Avenue West Hartford, CT 06117



April 7, 2017

Dear Cell Biology Faculty, Postdocs, Students, Staff, and Guests,

Welcome to the 2017 Cell Biology Retreat! Our event is being held at the Pond House Café in beautiful Elizabeth Park, America's oldest public rose garden. Opened to the public in 1897, Elizabeth Park is the botanical gem of the Hartford park system and is listed on the National Register of Historic Places. We hope you take time to enjoy a walk in the park!

There are a few things we would like to bring to your attention. Please note that <u>registration</u> <u>starts at 8:30am</u> and we request that <u>all posters are in place by 8:45am</u>. For oral talks, we will be providing a computer onto which we request all presenters to upload their presentations before the start of the talk sessions. <u>Morning talk presenters should upload their presentations on</u> <u>Monday the day before the retreat, by 3:00 pm (email to Tracy Uliasz)</u>. Afternoon presenters <u>should upload their presentations by 2:15pm on Tuesday</u>. Once registered, you will be provided with a copy of our program/abstract book and a name badge to wear throughout the event. Driving instructions are provided on the page following this letter. The majority of the event will be held indoors, in the ballroom near the Café entrance. However, weather permitting, our end-of-the meeting reception will be held on the patio.

If you are presenting a poster, the poster number listed in the header on the page of your abstract in this program is the number of the poster board on which you should place your poster. Presenters of even numbered posters should be present at their posters during the morning session (10:45-12:00). Presenters of odd numbered posters should be present during the afternoon session (1:15-2:30).

We will be recognizing the hard work of our students and postdocs who make a presentation by offering a travel award prize! The winner of this award will be determined by a raffle, and will be announced at the end of the day. Please note that you must be present to receive this award.

We hope that you enjoy this experience and that this event gives you the opportunity to discuss your work with your cell biology fellows.

Sincerely,

The Cell Biology Retreat Organizing Committee:

Rindy Jaffe	Leia Shuhaibar
Linda Shapiro	Ninna Shuhaibar
Guo Fong	Giulia Vigone
	Tracy Uliasz
	Deb Allaire

DRIVING DIRECTIONS FOR :

The Pond House 1555 Asylum Avenue West Hartford, CT 06117



Traveling East on Route 84

Take Rte. 84 exit # 44 (Prospect Ave.) At end of ramp, go through the stop sign to the traffic light, which is Prospect Ave.

Take left onto Prospect Ave. for (2) miles until it intersects with Asylum Ave. (the eight light).

Take left onto Asylum Ave. for (1/4) mile, take first left, which is Elizabeth Park entrance.

The Pond House is the first building on the left. Park along the roads or in the parking lot just past the Pond House, next to the Greenhouse.

From UConn Health

Take a right onto Farmington Ave. for (2) miles.

Take left onto Mountain Rd. for (3/4) mile.

Take right onto Fern St. for (1 ¹/₂) miles.

Take left onto Trout Brook Dr. for (3/4) mile.

Take right onto Asylum Ave. In (1) mile, turn right into Elizabeth Park.

The Pond House is the first building on the left. Park along the roads or in the parking lot just past the Pond House, next to the Greenhouse.

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Meeting Program 2017 Cell Biology Retreat

2017 Cell Biology Retreat April 18, 2017, Pond House, 1555 Asylum Ave, West Hartford, CT 06117

TIME	EVENT	EVENT		
8:30 – 9:00	Registration & poster	Registration & poster set up		
9:00 – 9:15	Welcome – Linda Shapiro Opening Remarks – Bruce Liang, Dean, School of Medicine			
9:15 – 10:30	Talks Session 1Moderator:N9:15 - 9:30N9:30 - 9:45F9:45 - 10:00N10:00 - 10:15F10:15 - 10:30L	/eneta Qendro Mayu Inaba Pat Murphy Mallika Ghosh Diana Golden ∟aura D'Angelo		
10:30 – 10:45	Coffee Break			
10:45 – 12:00	Posters – Session 1 (even numbers)			
12:00 – 1:15	Lunch & Flowers & Frisbee			
1:15 – 2:30	Posters – Session 2 (odd numbers)			
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3:45 – 4:00	Coffee Break/Take down posters			
4:00 – 4:05	Keynote Lecture Introduction – Henry Smilowitz			
4:05 – 5:00	Keynote Lecture Jim Hainfeld, President, Founder, and Chief Research Scientist Nanoprobes, Inc., Yaphank, NY "Biography of a Nanoparticle"			
5:00 – 5:10	Closing Remarks – Rindy, Linda, and Guo			

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Talks—Session 1

MT-nanotube mediated niche-stem cell signal specification

Mayu Inaba

Adult tissue stem cells produce highly differentiated but short-lived cells throughout life, contributing to the tissue maintenance and repair. Specialized environments called "niches" help to maintain stem cells by producing signals essential for stem cell maintenance. Stem cell-niche signaling has to be carefully regulated, since an excess of signal activation can lead to tumorigenic overproliferation of stem cells, while its shortage can deplete stem cells, causing tissue degeneration. Thus, niche signal has to meet two criteria 1) sufficient signal activation in stem cells, 2) no (or lower than threshold) signal activation in non-stem cells. Stem cells and their nonstem cell daughters are often juxtaposed each other, and thus how specificity of spatially confined niche signaling is achieved has been a mystery. It has been postulated that the secreted niche ligands "diffuse" only in a short-range, but how the range of diffusion can be tightly regulated remained unknown. We recently discovered previously unrecognized cellular protrusions, termed MT (microtubule-based)- nanotubes, that are specifically formed by stem cells and extend into the hub cells, the major niche component in the Drosophila testis. Our preliminary studies indicate that MT-nanotubes promote BMP signaling (Dpp ligand-Tkv receptor), a niche ligand required for stem cell maintenance. Based on our preliminary studies, we hypothesize that MTnanotubes function to mediate productive niche signaling such that only stem cells experience enough niche-dependent signal transduction, providing a mechanistic basis for the short-range nature of the niche signaling. We further explore molecular and cellular mechanisms of MTnanotube- mediated niche-stem cell signaling.

A Splice in Time: Alternative Splicing in the Endothelial Regulation of Flowinduced Arterial Inflammation

Patrick Murphy

Low and disturbed blood flow drives the progression of arterial diseases including atherosclerosis and aneurysms. The endothelial response to flow and interactions with recruited platelets and leukocytes determine disease progression. Here, we report widespread changes in alternative splicing of pre-mRNA in the flow-activated murine arterial endothelium in vivo. Alternative splicing was suppressed by depletion of platelets and macrophages recruited to the arterial endothelium under low and disturbed flow. Binding motifs for the Rbfox-family are enriched adjacent to the regulated exons. Endothelial deletion of Rbfox2, the only family member expressed in arterial endothelium, suppresses a large portion of the changes in transcription and RNA splicing induced by low flow. Our data reveal an alternative splicing program activated by Rbfox2 in the endothelium on recruitment of platelets and macrophages and demonstrate its relevance in transcriptional responses during flow-driven vascular inflammation.

CD13 is a critical regulator of β 1 Integrin recycling, cell migration and focal adhesion turnover

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¹Center for Vascular Biology, Department of Cell Biology, UCONN Health Center, Farmington, CT

CD13 is a multifunctional cell surface peptidase that is constitutively expressed on a variety of cells including myeloid cells, epithelial cells and endothelial cells activated at sites of angiogenesis and inflammation. Collectively, our recent studies demonstrated that upon Src-phosphorylation, CD13 regulates dynamin-mediated internalization of receptors of disparate classes to control downstream signal transduction pathways, implicating a fundamental role for CD13 in cellular endocytic processes. In the present study, we chose to explore a potential endocytic role for CD13 in the context of the wellcharacterized program of integrin trafficking. Integrins are trans-membrane receptors mediating interactions between the extracellular matrix (ECM) and the actin cytoskeleton via focal adhesions (FAs), which are dynamic structures that form and dissolve in tightly regulated steps to enable cell migration. We found that in Murine Embryonic Fibroblasts (MEFs), CD13 is localized with β1-integrin at FAs, the sites of communication between the ECM and the actin cytoskeleton and at cell-cell junctions in cancer epithelial cells, prompting our exploration of potential contributions of CD13 in FA turnover, integrin endocytosis and trafficking. Phenotypically, FAs in CD13KO fibroblasts are elongated and irregular with displaced FA accessory proteins, Vinculin, Paxillin and Talin with markedly reduced actin stress fibers and fewer microtubule extensions, indicating that FA formation and cytoskeletal organization is defective in absence of CD13. In accordance with this disrupted cytoskeletal organization in CD13KO cells, β1-integrin failed to cluster in response to ligand-coated beads and phosphorylation of FAK and Src was reduced, consistent with a link between CD13 and the control of FA dynamics. Importantly, we show that in WT MEFs, CD13 and β1-integrin co-internalize, traffic to EEA1+ and Rab5+ early endosomes and recycle to the plasma membrane together via Rab11a+ recycling endosomes. Conversely in CD13KO MEFs, internalized β1-integrin is again found in early endosomes but rather than recycling, integrin traffics from early endosomes to Rab7+ late endosomes/lysosomes. Pulse-chase assays confirmed that CD13 is necessary for β1-integrin recycling to the cell surface and that this process requires CD13 phosphorylation. Functionally, the absence of CD13 led to reduced cell spreading and cell-ECM migration in a wound-healing assay. While, CD13 accumulated at the cell leading edge and co-localized with IQGAP1 and Paxillin, deficiency of CD13 led to reduced accumulation of these proteins at the migrating front, suggesting that CD13 is necessary for recruitment of IQGAP1 at the leading front to regulate directional migration.

Mechanistically, we show that CD13 directly associates with the recycling regulatory protein ARF6 GTPase to promote integrin receptor surface expression. Since, autophagy is known to modulate focal adhesion turnover and cellular motility, we showed that CD13 and β 1 integrin associates with autophagosomal marker LC3, to regulate autophagy in migrating cells. This study defines a critical role for CD13 in controlling focal adhesion dynamics and actin cytoskeletal organization in association with focal adhesion kinases, FAK and Src and β 1 integrin recycling in association with GTPase ARF6 and its regulator IQGAP1, thereby directing the fundamental cellular processes of β 1 integrin trafficking, signal transduction, cell motility and autophagy.

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Lipoprotein SCARB1 Intronic Variant Associated with Increased Coronary Heart Disease Risk Affects Cardio-protective Gene Networks

Diana Golden*, Sameet Mehta[⁺], Antonina Kolmakova*, Annabelle Rodriguez* *Center for Vascular Biology, University of Connecticut Health Center, Farmington CT, USA +Yale Center for Genome Analysis, Yale University, New Haven CT, USA

Introduction: We previously reported a common intronic *SCARB1* (12q24.31) variant, rs10846744, located in an enhancer region, to be significantly associated with coronary artery disease (CAD) in the Multi-Ethnic Study of Atherosclerosis (MESA). RNA-Seq showed expression of the immune checkpoint inhibitor lymphocyte activation gene 3 (*LAG3*, 12p13.31), to be 5-fold lower in carriers of the risk allele, while low plasma LAG3 protein levels were also significantly associated with increased CAD risk in MESA, after multivariate regression analysis.

Hypothesis: That the *SCARB1* rs10846744 variant disrupts long-range transcriptional regulation of *LAG3* disturbing cardio-protective and anti-inflammatory gene networks to promote CAD.

Methods and results: Using functional genomics (HiC global chromatin capture, ChIP-Seq and RNA-Seq) in reference and risk EBV-transformed B lymphocytes to assess 3D chromatin architecture and gene-gene interactions at a 2.5kb resolution, we did not observe direct chromatin contacts between *SCARB1* and *LAG3*. In the reference allele, an enhancer-rich intermediate contact (12q13.13) was found containing genes associated with cholesterol (*SOAT2*) and NR2F2 signaling (*RARG*). This same 12q13.13 region was in direct contact with 22q12.3 (*APOLI*), an apoprotein associated with HDL and innate immunity. Micro-looping within the rs10846744 12q24.31 region showed direct contacts with other enhancers (*NCOR2*) and cardiovascular loci (*TMEM132B*), while *LAG3* micro-looping on 12p13.31 was associated with immune regulatory networks (*CD4*). Loci associated with viral infection, cytokine production, heart failure and autoimmunity were also identified. NR2F2 disrupted contacts in the risk allele, implicating NR2F2 as a dysfunctional rs10846744 transcriptional repressor altering gene networks. The risk allele included contacts near *PCSK9*, *VLDLR* and 2q33.1, a CAD locus.

Conclusion: Functional genomics of the *SCARB1* rs10846744 enhancer region identified a number of intra- and inter-chromosomal chromatin contacts in reference cells that were markedly disrupted in risk cells. Perturbing NR2F2 and/or genes disrupted in the *SCARB1*-NR2F2 immuno-cardiovascular axis may protect against CAD in the rs10846744 risk population.

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The Switch from a cAMP-independent to a cAMP-dependent Prophase 1 Arrest is Associated with Early GPR3 Expression and Activity in Mouse Oocytes.

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Mammalian oocytes are arrested in prophase I from around the time of birth, until a preovulatory luteinizing hormone (LH) surge that occurs after puberty. Small, growing oocytes are not competent to mature into fertilizable eggs because they do not possess adequate amounts of cell cycle regulatory proteins, particularly cyclin-dependent kinase 1 (CDK1). As oocytes grow, they synthesize CDK1 and acquire the ability to mature. After oocytes achieve meiotic competence, meiotic arrest at the prophase stage is dependent on high levels of cAMP that are generated in the oocyte by the constitutively active G_s-coupled receptor, GPR3. In this study, we examined the switch between GPR3-independent and GPR3-dependent meiotic arrest. We found that the ability of oocytes to mature, as well as CDK1 levels, were dependent on follicle size. Gpr3 was expressed and active in incompetent oocytes within early stage follicles, well before cAMP is required to maintain meiotic arrest. Oocytes from Gpr3^{-/-} mice were less competent to mature than oocvtes from Gpr3^{+/+} mice, as assessed by a germinal vesicle breakdown time course. However, CDK1 levels increased in *Gpr3^{-/-}* oocytes as follicles grew in diameter. These results demonstrate that cAMP in oocytes promotes meiotic competence, likely through the activity of GPR3. However, GPR3 activity is not likely to be associated with CDK1 levels, indicating that other proteins are responsible for the acquisition of meiotic competence. Corresponding email: Idangelo@uchc.edu

Ultrastructural analysis of the mouse ovarian follicle by serial section electron microscopy

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Somatic cells in the ovarian follicle have direct cell to cell contact with the oocyte throughout follicular development. As the oocyte grows in response to gonadotropins, it secretes the zona pellucida, which creates a physical barrier between the oocyte and the surrounding somatic cells. Cumulus cells remain connected to the oocyte through cytoplasmic projections known as transzonal projections (TZPs), which transverse the zona pellucida and make gap junctions and adherens junctions with the oocyte surface.

We used serial section electron microscopy to study the three-dimensional organization of TZPs at nanometer resolution on preovulatory mouse follicles. We were able to track and reconstruct single TZPs sent by several cumulus cells and characterize their interactions with each other and with oocyte microvilli, their connection (or lack of) with the oocyte, and visualize the abundance of TZPs with cargo or organelles inside them. On a larger scale, we looked at cumulus cells not directly adjacent to the oocyte but instead located on the second and third cell layers to determine an average approximation of how many of these cells are physically connected to the oocyte via TZPs.

Using the same serial section electron microscopy technique, we found that mural granulosa cells in mouse and rat preovulatory follicles have cytoplasmic projections that resemble TZPs morphologically but are oriented toward other mural granulosa cells with no apparent preferred orientation. These projections are thin (~150 nm diameter) and approximately 1-2 cell diameters in length (10-20 um). They frequently touch or invaginate neighboring cells and can be seen as single projections or in small bundles. Interestingly, unlike mural granulosa cells, cumulus cells do not have projections directed toward other cells but instead only have projections oriented toward the oocyte, suggesting an involvement in signaling of oocyte-derived factors.

Stimulation of microtubule-based transport by nucleation of microtubules on membrane organelles

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Microtubule (MT)-based transport can be regulated through changes in organization of MT transport tracks, but the mechanisms that regulate these changes are poorly understood. In *Xenopus* melanophores, aggregation of pigment granules in the cell center involves their capture by the tips of MTs growing toward the cell periphery, and granule aggregation signals facilitate capture by increasing the number of growing MT tips. This increase could be explained by stimulation of MT nucleation either on the centrosome or on the aggregate of pigment granules that gradually forms in the cell center. We blocked movement of pigment granules to the cell center and compared the MT nucleation activity of the centrosome in the same cells in two signaling states. We found that granule aggregation signals did not stimulate MT nucleation activity correlated with the recruitment to pigment granules of a major component of MT nucleation templates γ-tubulin, and was suppressed by γ-tubulin inhibitors. We conclude that generation of new MT transport tracks by the concentration of the leading pigment granules provides a positive feedback loop that enhances delivery of trailing granules to the cell center.

Vladimir Rodionov, rodionov@uchc.edu

The Regulation of Lactate Metabolism In The Context Of EMT Of Breast Cancer Cell Lines

Denisse Tafur-Cotrina¹, Patrick Svrcek², Bruce White¹, and Jeffrey Hoch³ ¹Department of Cell Biology, ²University of Connecticut School of Medicine, ³Department of Molecular, Microbial, and Structural Biology University of Connecticut Health, Farmington, CT

In breast cancer, an important distinction that affects prognostic outlook for patients is whether a cancer is *in situ* or has become invasive. To understand this analogously at a cellular level, masses in situ are akin to epithelial breast cancer cells and invasive masses are epithelial breast cancer cells that have become *mesenchymal* in phenotype, allowing the cells to effectively intravasate from their site of origin. These two phenotypes are linked by a process called Epithelial-to-Mesenchymal Transition (EMT). Our research focused on key differences between these two phenotypes in terms of lactate metabolism. Lactate is critical due to its role in the classic Warburg effect seen in a cancer metabolism and its usage as a building block and signaling molecule for rapidly dividing cells. Lactate metabolism was explored through endogenous cell surface membrane lactate receptor gpr81, which regulates the expression of lactate transporters and subsequent intracellular lactate levels. The two cell lineages used in our research are the standardized epithelial breast cancer cell line MCF-7 and BT-474; and its EMT-induced mesenchymal variant LMS and BTM respectively. All four cell lines were subjected to 3-D growth culture and converted into lysates for guantitative Nuclear Magnetic Resonance analysis (qNMR) to evaluate intracellular metabolite levels. We have determined that epithelial phenotype has a significant fourfold increase in intracellular lactate compared to the mesenchymal phenotype. While all of these cell lines undergo the Warburg effect, epithelial and mesenchymal have different isotypes of the MCT transporter. MCF-7 and BT-474 epithelial cells that form spherical tumor colonies in 3D culture, express high levels of MCT1 (lactate importer). In contrast, LMS and BTM mesenchymal cells form loose connective networks and lost expression of MCT1 while retaining high levels of MCT4 (lactate exporter). We have found a key regulator of these transporters to be gpr81, which upon knockdown, severely reduce the levels of MCT1 in MCF-7 and subsequently showed reduction in intracellular lactate by up to 50%, implicating gpr81 regulation of MCT transport of lactate. This Knockdown of gpr81 in MCF7 lines also reduced cell proliferation (p < 0.01).

VARIETIES OF PAIN AND THEIR TREATMENT Alan Fein

Nociceptive pain and its treatment

Neuropathic pain and its treatment

Pain in the central nervous system

Targeting VEGF-induced Calcium Transients to block Pathological Vascular Permeability via PLC-beta and TRP channel functions

Kevin Claffey, PhD.

Therapeutic treatment for acute vascular occlusion events such as heart attack and stroke necessitate opening blocked vessels and reperfusion of ischemic tissues. The acute reperfusion, combined with the initial tissue hypoxia, induces significant vascular permeability, tissue edema, damaging reactive oxygen, persistent interstitial edema and progressive cell damage and death. The extensive vascular permeability and edema accompanying the ischemia/reperfusion events are indicative of pathological permeability, and the prolonged nature of this event contributes to increased tissue damage, cell death with reduced function, and risk of eventual heart failure and death in some patients. We have an innovative proposal that outlines several key mechanisms promoting the induction and maintenance of pathological permeability. These include the response to the hypoxia-induced vascular endothelial growth factor (VEGF) in the affected vessels. The extent of the VEGF-induced vascular permeability is dictated by vascular endothelial cell balance of phospholipase C beta family members and the subsequent activation of membrane TRP family calcium channels. We have strong supporting evidence that inhibition of PLC_{β2} isoform can reduce vascular sensitivity to VEGF, and that inhibition of the TRPM2 channel downstream of VEGF activation, can ameliorate the pathological tissue edema, reduce cardiac lesion formation and improve heart function. To address the unmet clinical need to retain tissue function after ischemia/reperfusion injury, we have identified lead inhibitor compounds targeting both the PLCβ2 and the TRPM2 nodes in this pathway. This proposal will test the efficacy and potential benefit of PLCβ2 and TRPM2 inhibition using cell systems, and animal models with selective genetic deletions, as well as develop several classes of therapeutic inhibitors to this process. It, it is our hypothesis that; endothelial cell sensitivity to VEGF is dynamic and is controlled by PLC-beta isoform signaling, PIP2 substrate availability and downstream activation of TRP channels such as TRPM2 in acute ischemia/reperfusion injury. Targeting the vascular permeability events is highly significant due to its contribution to stroke and heart attack, poor outcomes and involvement of two interrelated mechanisms. Development of effective therapeutics can be applied to cardiovascular disease with ischemic events, thus improving quality of life, reduced health care costs and a significant improvement in mortality.

Redox Dependent Barrier Function of the Retinal Pigment Epithelium and Its Modulation by VEGFR-1

Yida Jiang, Li-Juan Duan, and Guo-Hua Fong Center for Vascular Biology and Department of Cell Biology University of Connecticut Health Center 263 Farmington Ave, Farmington, CT 06032

Retinal edema is serious ocular condition which may lead to loss of vision. While anti-VEGF therapy has been used to control retinal edema in patients with diabetic retinopathy or age-related macula degeneration (AMD), a large percentage of patients do not respond, and those who do initially may develop retinal degeneration in the long term due to non-vascular roles of VEGF in retinal neural tissues. Importantly, retinal edema is not only caused by leakage through capillaries to which anti-VEGF is effective, at least in diabetic retinopathy it is also substantially contributed by increased permeability through the retinal pigment epithelium (RPE). In contrast to our understanding on vascular permeability, much less is known on how the permeability of the RPE is regulated, and even less so how to target it for the purpose of restoring its barrier function. In this study we examine how RPE barrier function is compromised by pathologically relevant events and attempt to identify potential molecules which may be targeted for treatment. We found that treatment of cultured RPE cells or mice with an oxidative stressing molecule (NaIO3) may cause the breakdown of the RPE barrier. Consistent with a role of the anti-oxidant system in protecting RPE barrier function, mice deficient in Nrf2, a transcription factor whose normal function is to activate the expression of antioxidant enzymes, had increased permeability across the RPE. Since Nrf2 is a broadly expressed nuclear protein, its accessibility and tissue specificity may be limited for therapeutic targeting. Thus, we wondered if any cell surface molecule with less broad expression pattern might also regulate RPE barrier function and therefore provide a more accessible and specific therapeutic target. To this end, we performed siRNA-mediated knockdown of VEGFR-1 and VEGFR-2 in cultured RPE cells, and found that VEGFR-1 may indeed play an important role in maintaining RPE tight junctions under oxidative stress. Taken together, our data provide the first in vivo example of a role of the anti-oxidant system in protecting the RPE barrier function, and imply that VEGFR-1 might be a putative target for protecting RPE barrier function under oxidative stress.

Oxygen Coordinates Mutually Dependent Development of Retinal Astrocytic and Vascular Networks

Li-Juan Duan, Sarah Pan, and Guo-Hua Fong

Center for Vascular Biology and Department of Cell Biology University of Connecticut Health Center 263 Farmington Ave, Farmington, CT 06032

Retinal astrocytes are intimately associated with the vasculature, and are thought to play important roles in regulating retinal angiogenesis, vascular stability, and vascular permeability. Astrocytic abnormalities are associated with retinal diseases such as diabetic retinopathy. The biology of retinal astrocytes, however, remains poorly understood, limiting our ability to develop astrocyte-based therapeutic strategies. This study focuses on the relationship between the astrocytic and vascular networks during development. While it has been long accepted that retinal astrocytic network forms a template onto which the vascular network forms, our recent data indicate the opposite is true. While the astrocytic progenitor cells (APCs) do migrate from the optic nerve head (ONH) towards the retinal periphery ahead of the retinal vascular network, they mature into an astrocytic network pattern only in response the formation of vascular network development. Veqfr-2 to In flox/flox/Cdh5CreERT2 mice, tamoxifen dependent Vegfr2 knockout after birth not only blocked retinal vascular development, but also prevented the formation of the retinal astrocytic network while causing the accumulation of APCs, suggesting that astrocytic maturation is likely induced by signal(s) from the blood vessels. One likely candidate is oxygen. Indeed, when the oxygen sensing protein PHD2 (prolyl hydroxylase domain protein-2) was selectively knocked out in APCs, maturation of the APCs into mature astrocytes is hindered. By contrast, APC specific knockout of HIF-2a, a transcription factor which degrades by an oxygen and PHD2 dependent mechanism, accelerated APC maturation. Consistent with these genetic manipulations, direct exposure of neonatal mice to high levels of oxygen indeed accelerated astrocyte maturation. In conclusion, while retinal astrocytic progenitors induce angiogenesis, the oxygen from blood vessels induces the APCs to mature into the astrocytic network. Because mature astrocytes are non-proliferative whereas APCs are highly proliferative, the ability to main APC status by targeting PHD2 may provide therapeutic options to restore retina astrocytic abundance in diabetic retinopathy and therefore stabilize vascular structures.

Protecting Retinal Microvessels by Suppressing the Oxygen Sensing Mechanism in Astrocytes

Li-Juan Duan and Guo-Hua Fong

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Loss of retinal microvessels is an early stage phenotype of diabetic retinopathy. In addition, retinopathy of prematurity (ROP) is also preceded by massive loss of retinal microvessels before the onset of neovascularization. Early stage prevention of retinal microvascular obliteration may be beneficial in preventing or slowing down the progression of these diseases but current options are very limited. Given the suspected role of retinal astrocytes in mediating retinal vascular integrity, and our recent finding that the astrocytes are very sensitive to oxygen-induced differentiation from proangiogenic progenitors to nonangiogenic mature astrocytes, we hypothesized that perhaps their role in protecting vascular stability may be better maintained by targeting PHD2. To test this hypothesis, we initially induced global Phd2 knockout using Rosa26CreERT2 and subjected these mice to high levels of oxygen. Our data demonstrated that PHD2 deficiency protected retinal microvessels from oxygen-induced obliteration. The improved survival was associated with increased HIF-1a and HIF-2a levels in PHD2 deficient mice. When such mice were subject to Type 2 diabetes induced by a combination of streptozotocin (STZ) high fat diet, microvessels were more resistant to diabetes-induced damages than floxed controll littermates, displaying reduced numbers of acellular capillaries and ghost pericytes. To further examine if such protective effects originated from astrocytes, we generated astrocyte specific PHD2 knockout mice. These mice also displayed improved vascular stability under both hyperoxia and diabetic conditions. In contrast to PHD2 disruption, targeted disruption of HIF-2a alone or both HIF-1a and HIF-2a together led to diminished vascular stability even under normal housing conditions indicating the critical role of HIF-a especially HIF-2a in maintaining retinal vascular stability. Taken together, our data suggest that the astrocytic oxygen sensing mechanism may be a novel preventive target for early stage diabetic retinopathy and retinopathy of prematurity.

Title: **Monocyte TRPM2 channels mediate inflammation in cardiovascular disease** Authors: Albert S. Yu, Zhichao Yue, Lixia Yue Presenting Author: Albert S. Yu (<u>alyu@uchc.edu</u>) Affiliations: UConn Health, Farmington, CT, USA Presentation Preference: Oral Presentation

Abstract:

Introduction/Background: It is estimated that by 2030, 43% of US adults will have some form of cardiovascular disease (CVD). Coronary heart disease is a leading contributor to this statistic and arises when underlying systemic atherosclerosis manifests as blockage of the coronary arteries. While improved treatments restore blood flow after an acute episode of myocardial infarction (MI), the likelihood of adverse events and mortality remains high. Previous studies have shown that Transient Receptor Potential Melastatin 2 (TRPM2), a calcium permeable ion channel expressed in immune cells, exacerbates inflammation and reduces heart function after MI.

Methods: To study MI in a clinically relevant setting, ApoE-deficient mice were utilized to obtain systemic inflammation. As monocytes are key mediators of inflammation in CVD, we hypothesized that Trpm2-knockout (Trpm2-ko) reduces inflammatory markers in the circulating monocyte population. In addition, we hypothesized that Trpm2-ko reduces aortic plaque formation, a key factor in the progression of CVD, and improves heart function following MI. Furthermore, we assessed whether TRPM2 influences the circulating monocyte population in wt and Trpm2-ko mice. TRPM2 expression on monocytes/macrophages was assessed using patch-clamp electrophysiology. Peripheral blood monocytes were characterized by flow cytometry using various markers including Ly6C. Atherosclerosis plaque was guantified by Oil Red staining of the aorta. MI followed by reperfusion was induced using standard techniques heart function measurements and were obtained using echocardiography and P-V loop recordings.

Results: Higher TRPM2 expression was observed in ApoE-deficient mice fed with a high fat diet (HFD) compared to standard chow. Although peripheral blood analysis showed similar levels of circulating inflammatory Ly6Chi monocytes between wt and Trpm2-ko mice (n=12), Trpm2-ko mice had an elevated level of circulating anti-inflammatory Ly6Clo monocytes. Moreover, *Trpm2* deletion significantly reduced atherosclerosis as demonstrated by reduced lesion area. Finally, Trpm2-ko mice showed improved heart function two months post-MI.

Conclusions/Future Directions: Our results show that the generation of a systemic inflammatory state may lead to an increase in TRPM2 expression in circulating monocytes/macrophages. As Trpm2-ko leads to an increase in the population of antiinflammatory Ly6Clo monocytes, it is likely that the loss of TRPM2 contributes to the reduced atherosclerosis and improved heart function after MI. Future studies will be focusing on monocyte specific *Trpm2* deletion to reveal the mechanism of protection. As the burden of CVD will grow substantially over the next decade, identifying a target such as TRPM2 to reduce atherosclerosis and improve heart function after MI will have a pivotal impact on the health of the US population.

Poster No.5 Regulation of Ryanodine Receptor mediated perinuclear calcium by the mAKAP complex

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Hypertrophic cardiomyocytes are characterized by their inability to properly regulate intracellular calcium, an ion essential for contraction, homeostasis, and activation of transcription factors. In diseased cardiomyocytes, calcium releases impacting transcription factors are segregated from those necessary for excitation contraction coupling. Hypertrophic signaling results in large pools of calcium collecting in the nucleus and activating various diseaseinducing transcription factors. A key player in these signaling events is a scaffold protein known as muscle A-Kinase Anchoring Protein (mAKAP). mAKAP binds and recruits various proteins including protein kinase A (PKA) and the ryanodine receptor (RyR2) to spatiotemporally coordinate hypertrophic signaling events surrounding the nucleus leading to nuclear calcium buildup. While a multitude of work has implicated mAKAP in the development of heart disease and up-regulation of hypertrophic genes, the source of calcium modulated by mAKAP is unknown. Our central hypothesis is that stimulation of the cAMP pathway and activation of bound PKA will phosphorylate bound RyR2 leading to release of calcium in this region. This calcium would be pumped into the perinuclear space, ultimately leading to hypertrophic gene expression. Using a single cell microscopy technique, we measured calcium levels under stimulation and inhibition in this specific cellular region. Our results show RyR2 releases calcium surrounding the nuclear envelope, and that activation of PKA is needed to stimulate this release under physiological conditions. These experiments provide evidence that the ryanodine receptor is responsible for releasing calcium surrounding the nucleus that results in activation of hypertrophic transcription factors.

Synergistic activities of multiple cyclic AMP phosphodiesterases prevent premature meiotic progression, ovulation and progesterone signaling in mouse ovarian follicles.

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In the mammalian ovary, cAMP mediates most of the cellular responses to luteinizing hormone (LH). Therefore, to avoid premature responses, cAMP concentration must be finely regulated. Regulation occurs through control of adenylyl cyclases and phosphodiesterases (PDEs), but the underlying mechanisms are not fully understood. Inhibitors of PDE4 have been reported to cause resumption of meiosis in follicle-enclosed oocytes of rats and mice (1, 2), suggesting that PDE4 might play an important role in controlling cAMP levels prior to stimulation by LH. However, among the cAMP PDEs expressed by preovulatory granulosa cells in rat, mouse and human, PDE7 and PDE8 show the highest mRNA levels, with lower expression of PDE4 (3-5). Also, in various cell types, such as Leydig cells and adrenocortical cells, multiple PDEs have been shown to act synergistically in the regulation of steroidogenesis (6-8). This raises the question of whether PDE4, PDE7 and PDE8 act synergistically to prevent spontaneous meiotic resumption and ovulation. To address this question, we used specific inhibitors of PDE4 (Rolipram, Tocris BioScience), PDE7 (Pfizer) and PDE8 (PF-04957325, Pfizer) and we tested their effects, alone or in combination, on meiotic resumption and ovulation.

Both of these responses were partially stimulated by PDE4 inhibition alone, with no effect of PDE7 and PDE8 inhibition. However, when the 3 inhibitors were applied together, both meiotic resumption and ovulation occurred at rates much higher than seen with PDE4 inhibition alone. These findings indicate that synergistic cAMP phosphodiesterase activities of PDE4, PDE7 and PDE8 prevent premature meiotic progression and ovulation.

A key regulator of the ovulation process is progesterone, which is produced by granulosa cells, and acts by binding and activating its nuclear receptor PGR. Both the expression of PGR and progesterone production are induced by LH. Using the PDE4, PDE7 and PDE8 inhibitors, we investigated the role of different cAMP PDEs in the regulation of progesterone signaling. While a synergistic action of PDE4, PDE7 and PDE8 is required for preventing the premature expression of PGR, the 3 PDEs contribute additively, but not synergistically, to preventing spontaneous progesterone production, with PDE4 playing the major role. Together, our results suggest that the combined activity of these 3 different cAMP PDEs in granulosa cells suppresses fluctuations in cAMP that could cause premature responses in the ovarian follicle.

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Ondontoblast processes in developing teeth investigated by serial section electron microscopy

Ninna Shuhaibar and Mark Terasaki

The tooth is composed mostly of dentin, the highly mineralized collagenous matrix that supports the enamel. In contrast to the non-living enamel, dentin undergoes growth throughout life and is essential for limiting damage to and protecting the tooth pulp. The odontoblasts are the cells that form and maintain the dentin. They are not located within the dentin, but instead form a layer at the boundary between the pulp and the dentin. The odontoblasts deposit the predentin and subsequently mineralize it around thin, branching cellular processes that extend from the distal end of the cell body throughout the dentin. These are called odontoblast processes, and are present within a dentinal tubule. There are many things about these remarkable processes that are not well understood. How exactly do they accomplish dentin formation? Do they sense damage? Are they coordinated with nerves that enervate the dentin? How do the odontoblast and their processes interact with the nerves? Some of these issues could be addressed with more knowledge of the fine structure of the three-dimensional organization of the odontoblast processes and dentinal tubules. Recently, a new method of serial section electron microscopy was developed that allows 3-D reconstruction of microscopic cellular structures (Terasaki et al., 2013; Kasthuri at al., 2015). We propose to use this new technology to investigate the organization of odontoblast processes, dentinal tubules and nerve fibers in dentin.

Proximal tubule proteins as urinary biomarkers of early renal damage in murine models of obstruction and children with congenital UPJO

Emily Shearier¹, Claire Gerber¹, Miriam Harel², Robin Lo¹, Fernando Ferrer², Linda Shapiro¹ ¹Center for Vascular Biology. UConn Health ²Pediatric Urology Associates Ureteropelvic junction obstruction (UPJO) is a form of congenital nephropathy that affects 1 in 500 children and can vary in clinical manifestation¹. The blockage of ureters due to UPJO can results in a wide range of damage, with surgery reserved for those patients with the greatest loss of renal function. Currently, it is difficult to diagnose the level of damage occurring during the blockage to determine when surgical intervention is required. This is done using serum creatinine levels, which remain unchanged until 50% of kidney functional units are lost, and often-inconclusive renal scans. Identification of reliable protein biomarkers present in UPJO patient urine are may potentially provide an earlier and more reliable diagnosis, and would be non-invasive. A number of biomarkers have already been proposed, but these biomarkers are proteins produced in response to injury (NGAL, IL-8, KIM-1) and therefore the accuracy varies with the etiology of the renal damage². During presentation of UPJO, the blockage of the ureter leads to a backpressure within the kidney, damaging the delicate structures including the proximal tubule. Within each proximal tubule exists a brush border, composed of apically expressed proteins shed due to pressure from the blockage. In particular, proteins containing a single pass within the proximal tubule membrane are more likely to be shed, which is the focus of this study. Based on proteomic analysis of brush border lysates, three proximal tubule metallopeptidases (CD10, CD13, and CD26) were chosen. Bladder urine samples from 12 UPJO patients and 12 control samples were tested for levels of NGAL, KIM-1, CD10, CD13, and CD26 by ELISA. Each of the metallopeptidases outperformed the previously published biomarkers, with the levels being significantly higher in the UPJO patient samples compared to the control³. An additional 14 UPJO samples have since been attained, further demonstrating the significance. Because in human patients it is rare for a biopsy to be performed on the renal tissue, it is difficult to make a direct comparison between urine biomarker levels and the levels of damage. To assess this relationship fully, the mouse model of unilateral ureteral obstruction (UUO) was utilized. Urine and kidneys were collected at 2, 3, 4, 5, 7, 10 day (s) post-ligation from both the ligated and unligated kidney. Biomarkers as described above were analyzed using ELISA, and damage quantified using histological techniques including H&E, Trichrome, PAS, Lotus lectin, and ApoTag. Damage increases with time in all measurements, and was synthesized into a single score. These scores were compared to each ELISA biomarker measurement, showing the predictability of each with increasing damage, and further supported the hypothesis CD10, CD13, and CD26 are reliable biomarkers of the associated damage with UPJO.

Serial section immunogold electron microscopy of phosphorylated connexin 43 in ovarian granulosa cells

Rachael Norris, Valentina Baena, and Mark Terasaki

Gap junctions comprised of Connexin43 (Cx43) connect the granulosa cells of ovarian follicles in mammals. In response to luteinizing hormone, which triggers the resumption of meiosis, there are marked changes in Cx43 phosphorylation and an increase in internalized gap junctions (or connexosomes) is detected by electron microscopy. Results from Western blotting and immunofluorescence studies indicate that Cx43 phosphorylation is associated with gap junction internalization. To determine the precise localization of specifically phosphorylated Cx43, electron microscopy (EM) provides the best possible resolution. Further, serial sections of electron micrographs are needed to determine the whole structure of a gap junction, since invaginating gap junctions may appear to be fully internalized gap junctions in a single section. Here, we adapted a method used for 3D electron microscopy to work with immunogold labeling. In this manner, ultrathin tissue sections are collected on tape with an automatic tape collecting ultramicrotome (ATUM). The sections on tape are attached to a silicon wafer, then imaged by scanning EM. With this technique we can discern the full structure of a gap junction, an invaginating gap junction or connexosome. We labeled serial sections of preovulatory ovarian follicles with antibodies against either total Cx43, pS262 Cx43, or pS368 Cx43. We found that Cx43 is phosphorylated on S368 (a protein kinase C site) in both gap junctions and connexosomes. In contrast, Cx43 is phosphorylated on S262 (a MAP kinase site) only in some connexosomes. Our results suggest that MAP kinase phosphorylation of Cx43 may play a specific role in gap junction internalization or in connexosome processing in ovarian granulosa cells.

The mAKAP complex orchestrates the dephosphorylation of MEF2D in muscle cells to stimulate its activity

Shania N. Aponte Paris, Michael S. Kapiloff, Kimberly L. Dodge-Kafka

Myocyte Enhancer Factor 2D (MEF2D) is a transcription factor required for the development of pathological remodeling as well as the induction of cardiac hypertrophy by pressure overload. However, little is known about how this transcription factor is regulated. Previous work showed that the scaffolding protein mAKAP (muscle A-Kinase Anchoring Protein) orchestrated the calcineurin (CaN)-mediated stimulation of MEF2D in skeletal muscle cells. CaN and mAKAP immunoprecipitate with MEF2D in skeletal muscle cells and primary neonatal cardiac myocytes (RNV); MEF2D and CaN association was dependent on mAKAP expression. Furthermore, expression of anchoring disrupting peptides, which displaced CaN binding to mAKAP, blocked the induction of MEF2D gene transcription in skeletal myoblasts as well as the induction of cardiac hypertrophy in RNV, demonstrating the importance of the mAKAP complex for stimulation of MEF2D activity. The goal of this study was to elucidate the molecular mechanism that underlies the regulation of MEF2D activity by mAKAP-bound CaN. Stimulation of C2C12 cells with differentiation media induces the binding of active CaN to mAKAP, resulting in the dephosphorylation of MEF2D at Serine 444. Our current focus is on determining if Serine 444 dephosphorylation results in the desumovlation of MEF2D, allowing for increased gene transcription. Overall, our data suggests that the mAKAP complex focuses the actions of CaN onto MEF2D, to promote regulation of its post-transcriptional modifications, allowing for the increased transcriptional activity seen in both stimulated skeletal muscle myoblasts and stressed cardiac myocytes. Understanding the regulation and activity of the transcription factor MEF2D and its associated proteins is a crucial step towards the ability to block the development of cardiac hypertrophy.

A mathematical model of iron dynamics in a mouse

Jignesh Parmar & Pedro Mendes Center for Quantitative Medicine & Department of Cell Biology, UConn Health Abstract

Iron is indispensable for life, however, unliganded iron is toxic and has been implicated in the pathogenesis of several diseases such as diabetes and cancer. Kinetics of iron metabolism have been well studied experimentally at whole-body and tissue levels, but the complex interaction between absorption, distribution, storage and mobilization of iron and its regulation by hormones makes it difficult to understand intuitively. Mathematical models are used to integrate data and our knowledge to better understand the system, also generating testable predictions. Here, we developed a mathematical model of mouse iron physiology to gain insights into iron kinetics and its hormonal regulations. Model calibration against previously published data on radiolabeled iron distribution revealed an essential role of NTBI uptake by the liver under high iron diet condition. The model was validated by its ability to qualitatively simulate the pathophysiology of several iron disorders such as hemochromatosis, β -thalassemia and anemia of inflammation. The present model is a roadmap towards a more comprehensive mathematical model incorporating cellular and organs level details, which then can be used for generating new predictions as well as for exploring new therapeutics for various iron disorders.

Loss of CD13 Enhances Albumin Uptake in Renal Proximal Tubules

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Certain disease states, such as Diabetes Mellitus, damage glomerular function and result in albuminuria, which further promotes renal damage and may progress to chronic kidney disease or end stage renal failure.¹⁻⁵ Urinary proteins are normally efficiently resorbed via Megalin-Cubilin receptormediated endocytosis by the epithelial cells in renal proximal tubules.^{4,5} CD13 has previously been shown to be a negative regulator of endocytosis in other epithelial cell types.⁶ Here we show that albuminuria is reduced and albumin uptake is increased in CD13 KO mice *in vitro* and *in vivo* by *i*) increased uptake of FITC-albumin in isolated primary proximal tubule cells (PPT), ii) decreased clearance of serum FITC-albumin 12 and 24 hours post tail-vein injection, and *iii*) decreased urinary albumin levels following a 9-day albumin overload model. Furthermore, we show that CD13 KO PPT cells may attenuate the damaging effects of albumin through increased resorption *in vitro*. Bovine serum albumin treated CD13 KO PPT cells exhibited decreased cleaved caspase-3 levels while exhibiting increased albumin uptake compared to their WT counterparts. The underlying mechanism of CD13 regulating albumin uptake remains unknown. Western blot analysis of kidney homogenate and immunofluorescence of the renal cortex show equivalent levels of Megalin and Cubilin. Coimmunofluorescence studies of CD13 and Megalin do not reveal co-localization. We are currently investigating the relationship of CD13, albumin, and Rab proteins to pinpoint differences in endocytic vesicular trafficking between the CD13 KO and WT mice. Our results demonstrate that CD13 serves as a key novel mediator for albuminuria-induced proximal tubule damage and is a potential therapeutic target for preventing further renal disease progression to chronic kidney disease or end stage renal failure.

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CD13 deficiency leads to bigger and vulnerable atherosclerotic plaques

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Abstract

Atherosclerosis is an inflammatory cardiovascular disorder that affects millions of people worldwide. Understanding the underlying mechanisms leading to atherosclerosis will aid in developing efficient treatment strategies. CD13 is an aminopeptidase that is expressed on myeloid cells, activated endothelial cells and some epithelial cells. CD13 has been implicated to play a role in trafficking of immune cells to sites of injury and repair, homotypic adhesion as well as in angiogenesis. Based on this knowledge, we wanted to determine the role of CD13 in atherosclerosis. To this end, CD13+/+ and CD13-/- mice were crossed with LDLR-/- (low density lipoprotein receptor) mice to generate CD13+/+ LDLR-/- and CD13-/- LDLR-/- mice. CD13+/+ LDLR-/- and CD13-/- LDLR-/- mice were either fed regular diet or high fat diet for 9 weeks or 12 weeks or 15 weeks. At the end of different time points, mice were euthanized and aortic roots along with innominate arteries were analyzed for atherosclerotic lesions. At 9-week and 12-week time points, no difference was observed in the average lesion size between CD13+/+ LDLR-/- and CD13-/- LDLR-/- mice on high fat diet. But at the 15-week time point, CD13-/- LDLR-/- mice had bigger lesions in comparison to CD13+/+ LDLR-/- mice. Upon further examination, it was clear that the lesions in CD13-/- LDLR-/- mice had larger necrotic areas. This result was validated when we observed an increased number of apoptotic cells in CD13-/- LDLR-/- mice at the 15week time point. To understand the cellular mechanism, bone marrow derived macrophages (BMDMs) were generated from CD13+/+ and CD13-/- mice and were tested to determine their inherent potential to take up lipids. To this end, CD13+/+ and CD13-/- BMDMs were incubated with highly oxidized low density lipoprotein (oxLDL) for 24h and 48h. Subsequently, BMDMs were stained with Oil Red O to determine the extent of oxLDL uptake. Quantification of extracted Oil Red O stain revealed that the CD13-/- BMDMs had taken up more oxLDL than CD13+/+ BMDMs. Concomitantly, this increased uptake of oxLDL in CD13-/- BMDMs led to an increase in the number of apoptotic (24h) and necrotic cells (48h) as compared to CD13+/+ BMDMs at corresponding time points. This data suggested that the lack of CD13 led to an increased uptake of oxLDL which, subsequently caused higher apoptosis of BMDMs. This thereby explains the increase in the number of apoptotic cells as well as larger necrotic areas seen in the lesions of CD13-/- LDLR-/- mice. Further studies will be aimed at determining the molecular mechanisms of the differential uptake of oxLDL as well as the signaling pathways that lead lead to apoptosis in these cells. Additionally, it was also observed that the average ratio of fibrous cap thickness to the necrotic core area was slightly lesser in the lesions of CD13-/- LDLR-/- mice as compared to the lesions of CD13+/+ LDLR-/- mice. This would indicate that the lesions of CD13-/- LDLR-/- mice were of the vulnerable phenotype and are maybe prone to rupture than the lesions of CD13+/+ LDLR-/- mice.

Potential Role of TRPM7 in pressure overload induced heart failure

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ABSTRACT The transient receptor potential melastatin 7 (TRPM7) is a unique channel protein which processes both ion channel and protein kinase functions. TRPM7 is ubiquitously expressed in various tissues and cells, and plays a crucial role under a variety of physiological/pathological conditions, such as early embryonic development, anoxia induced neuronal cell death, cell proliferation and differentiation. We found that TRPM7 is highly expressed in cardiac fibroblasts and is the major Ca²⁺ permeable channel in cardiac fibroblasts. Here we show that in transverse aortic constriction (TAC) induced hypertrophy and heart failure mouse model, TRPM7 is highly up-regulated. Deletion of Trpm7 significantly increases heart performance in comparison with the wild type (WT) mice after TAC. The ejection fraction is significantly larger and the ratio of heart versus body weight is much smaller in TRPM7-KO mice compared to WT mice after TAC. Moreover, depletion of Trpm7 significantly decreases collagen production and fibrosis formation in TAC induced heart failure.

Cell type-specific roles of Leukemia Inhibitory Factor in regulating angiogenesis through modulation of the oxygen-sensing system

Sarah Pan, Li-Juan Duan, Guo-Hua Fong

Angiogenesis occurs in response to hypoxia, which is detected by a system involving prolyl hydroxylase domain-containing proteins (PHDs) and hypoxia-inducible factor (HIF). Using oxygen as a substrate, PHDs catalyze the hydroxylation of HIF α which leads to HIF α degradation. By increasing HIF α levels and expression of its pro-angiogenic targets including VEGF, hypoxia stimulates angiogenesis until the oxygen supply is normalized. While the oxygen-sensing mechanism of PHDs has been described in detail, regulation of PHD expression is not well understood. A better understanding of how the oxygen sensing system is regulated would potentially enable development of new strategies for treament of neovascular eve diseases. The pleiotropic cytokine leukemia inhibitory factor (LIF) has been shown to regulate angiogenesis in vitro and in vivo. Retinal vascular density is elevated in LIF germline null mice during vascular development but returns to normal by P8, suggesting that LIF transiently inhibits angiogenesis. LIF has been found to induce Phd2 expression in osteoclasts, and we observed a similar effect in primary astrocyte culture. Therefore, we hypothesized that LIF inhibits angiogenesis by upregulating PHD2 and decreasing HIFa levels. Surprisingly, in LIF^{f/f}/Rosa26CreERT2, Lif^{f/f}/Cdh5CreERT2, and Lif^{f/f}/GfapCre mice (postnatal tamoxifen-induced global KO, endothelial cell-specific KO, and constitutive astrocyte progenitor- and astrocyte-specific KO, respectively) retinal vessel branching and progression of the vascular front were reduced at P4, suggesting that LIF has a pro-angiogenic role. To explain the apparent contradiction between this data and the germline LIF-null data, we now hypothesize that LIF has distinct regulatory effects in different retinal cell types which promote or inhibit angiogenesis, and the timing of LIF signalling and downstream effects contributes to the angiogenic phenotype observed. Further work is required to test this hypothesis and determine whether the regulatory effect of LIF is mediated by PHD2.

AMPK Signaling is Advantageous to Breast Cancer Cells under Microenvironmental Stress

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Recurrent and metastatic breast cancers are responsible for the majority of breast-cancer related deaths. Breast cancer cells are able to adapt to stressors within the microenvironment including hypoxia, low nutrient levels, and increased levels of reactive oxygen species (ROS). Cancer cells can respond to these stressors via cell cycle inhibition, metabolic alteration, or increased antioxidant activity, and their survival is dependent on how they integrate the signals from their environment, and their subsequent response. AMPK (AMP-activated protein kinase) is the main metabolic sensor of the cell, and both its expression and activity have been reported to be altered in breast cancer. Moreover, there are two isoforms of the catalytic subunit (α 1 and α 2), which are believed to have differential functions. Using estrogen receptor-positive human breast cancer cell lines, we investigated the effect of differential AMPKα isoform expression on breast cancer cell survival. MCF-7 cells over-expressing AMPKa2 displayed increased survival under hypoxia treatment when grown as spheroids. In addition, BT474 cells transduced with an shRNA targeting AMPKa2, showed decreased survival as spheroids under low glucose conditions. Culturing these cancer cells as spheroids induces activation of the p38MAPK pathway, with stronger induction in the cells expressing AMPK α 2. Moreover, inhibition of p38 signaling impairs spheroid viability. One means by which AMPKa2 could be providing this survival advantage to breast cancer cells is through the modulation of ROS levels. We found that AMPKa2 expressing MCF-7 cells have basally lower ROS levels. Knockdown of the antioxidant protein catalase ablated this difference between AMPKα2 expressing and control MCF-7 cells. Moreover, MCF-7 cells which expressed AMPKa2 but not catalase showed minimal AMPK activation in response to microenvironmental stressors. This may indicate cross-talk between AMPK and antioxidant activity in breast cancer cells, and could potentially be mediated by p38MAPK. To evaluate this differential response to microenvironmental stress in vivo, MCF-7 cells expressing either GFP or AMPKa2 were injected into athymic nude mice previously implanted with slow-release estradiol pellets. After one week, the estradiol pellets were removed to induce a dormant or stress period for thirty days. When cell number was evaluated by H&E staining, twice as many AMPKa2 expressing cells were present as control cells following estradiol deprivation. Analysis of proliferation by Ki67 staining indicated that the GFP cells maintained proliferation during deprivation, while AMPKa2 cells did not. ApoTag staining revealed a similar trend for apoptotic cells. This suggests that an inability to control cell cycle resulted in a decreased survival of the GFP cells under estradiol deprivation. Following the deprivation period. estradiol pellets were re-implanted and residual dormant tumors resumed growth. AMPKg2 tumors grew to roughly double the size of GFP tumors. Interestingly, AMPKa2 tumors had a higher number of mitotic events than did GFP tumors. This could be due to more viable cells being present following estradiol deprivation. In conclusion, the additional expression of AMPKa2 appears to provide a survival advantage to breast cancer cells, specifically in stressful microenvironments. This survival advantage is possibly conferred by increased regulation of ROS levels.

Preclinical Evaluation of FTY720 in combination with Fenretinide in neuroblastoma Mei-Hong Li¹, Carol Bult², Linda H. Shapiro^{1*}, Fernando Ferrer^{3*}

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Abstract

Neuroblastoma (NB) is the most common extra-cranial solid tumor in childhood. Poor outcomes for children with advanced disease underscore the need for novel therapeutics. FTY720, an FDA-approved multiple sclerosis drug targeting sphingolipid signaling has demonstrated potent preclinical anti-cancer activity in various cancers including NB. Fenretinide (4-HPR) has also been shown to potentiate beneficial changes in sphingolipid species and is currently in NB clinical trials. We postulated that dual targeting of the sphingolipid pathway may improve efficacy and herein tested the novel combination of FTY720 and 4-HPR in NB and explored potential anti-cancer mechanisms. Compared to monotherapy, the combination therapy dramatically reduced NB cell viability, along with significant increases in the levels of three dihydrosphingolipids (dihydro-sphingosine-1-phosphate, dihydrosphingosine and dihydro-C16-ceramide). Identification of potential transcriptional targets by RNA-seq and Ingenuity Pathway Analysis indicated the combination therapy highly impacted cell cycle and its regulator *CDKN1A/p21*, which was confirmed at the gene expression level and functionally by flow cytometric demonstration of cell cycle arrest at the G1/S phase. Importantly, the combination therapy was also extremely effective in NB xenografts, causing a dramatic inhibition of tumor cell proliferation and increased apoptosis. Furthermore, by using exogenously-added dihydrosphingosine and the specific serine palmitovltransferase inhibitor myriocin, we established a positive relationship between sphingolipid metabolism and p21 signaling. Our data demonstrate that combination therapy with FTY720 and 4-HPR has potent anti-cancer activity against NB both in vitro and in vivo and indicates that targeting sphingolipid metabolism might be an effective alternative approach to dysregulating the cell cycle in NB.

Presentation Preference: Poster

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PSMA must be localized on vascular endothelial cells for pathologic angiogenesis to occur

Anisha Lewis, Danica Anukam, Christina Grant, Linda Shapiro, Leslie Caromile

Primary prostate cancer (PC) results in a highly vascular tumor arising from prostatic epithelial cells. It is known that tumor vascularity has a major impact on tumor growth and drug responsiveness through its effects on tumor blood flow, oxygenation, and the permeability of chemotherapeutic drugs. As a result, vascularity is an independent prognostic factor for many human tumors, with high vascular density often associated with poor prognosis following surgery or conventional chemo/radiotherapy. For example, increased vascular density in PC is associated with decreased patient survival.

In the tumor microenvironment, tumor cells are closely juxtaposed with angiogenic endothelial cells. Prostate Specific Membrane Antigen (PSMA) is a 750 amino acid type II transmembrane peptidase encoded by the folate hydrolase 1 (FOLH1) gene that is normally expressed predominantly in prostatic epithelium. Importantly, PSMA expression increases significantly as PC advances and correlates negatively with Gleason score. Interestingly, PSMA is upregulated on the angiogenic vasculature of most solid tumors and we have shown that PSMA stimulates extracellular matrix (ECM) dependent-integrin activation and the subsequent focal adhesion kinase/p21-activated kinases (FAK/PAK) signal transduction cascade to direct endothelial cell adhesion and invasion; thereby regulating angiogenesis. In further studies, we established that PSMA hydrolyzes fragments of the ECM protein laminin to generate pro-angiogenic peptides that increase $\alpha\beta1$ integrin activation and stimulate angiogenesis. Interestingly, we also demonstrated that PSMA promotes angiogenesis in the retina by a similar enzymatic mechanism.

Since the pro-angiogenic peptides that are generated by PSMA are in the extracellular space, it is possible that the increasing abundance of PSMA on tumor cells in advancing PC would be sufficient to promote angiogenesis when the vasculature lacks PSMA. Alternatively, we have recently demonstrated that the PSMA cytoplasmic domain dictates signal transduction pathways in PC tumor cells suggesting that cell-intrinsic expression of PSMA may be required for some of its functions. To directly address if endothelial cell intrinsic expression of PSMA is necessary for its angiogenic regulatory properties, we injected a syngeneic murine PC cell line that expresses high levels of extracellular PSMA into wild type and PSMA knockout mice. In this allograft model the tumor cells are PSMA positive while the vasculature is wild type or lacking PSMA. We found that despite high PSMA expression in the tumor microenvironment, angiogenesis is impaired if the vascular endothelial cells lack PSMA. Currently, a number of PSMA inhibitors and targeting antibodies have been developed which should target both the tumor cells and the angiogenic endothelium and may potentially improve PC therapeutic outcomes.

Absent CD13 in combination with a toll like receptor agonist can increase the efficacy of cancer vaccine adjuvants

Veneta Qendro, Mallika Ghosh, Linda Shapiro

Vaccines have been extraordinarily successful in the fight against human disease by exploiting the immune system to recognize and eliminate abnormal cells or pathogenic organisms. Recent advances in the research of cancer vaccines have led to the development of three FDA-approved anti-cancer vaccines for cervical, liver and prostate cancers. However, despite the great innovation these vaccines represent, they have proven limited to only prophylactic uses, with little to no therapeutic effects (extending patients' life by only a few months- prostate cancer).

Ongoing research in the field of cancer vaccines recognizes that poor immunogenicity is one of the main issues that prevents generation of reliable therapeutic cancer vaccines. Therefore major effort has been dedicated in the identification of efficient cancer adjuvants- the necessary vaccine components that enhance and activate the immune system.

Here we propose the combination of two such molecules, with individual properties that could potentially generate the ideal vaccine adjuvant.

MPLA (monophosphorylated lipid A), a synthetic analog of LPS shown to vigorously activate the adaptive immune response without triggering deleterious inflammatory consequences, has been recently approved as a component of the prophylactic, HPV-driven (Human Papilloma Virus) cervical cancer vaccine, Cervarix.

On the other hand, absent CD13 – a multifunctional cell surface peptidase constitutively expressed on all lineages of myeloid cells – has been shown to preferentially increase TLR4 endocytosis towards the same endocytic-signaling pathway triggered by MPLA. Additionally, absent CD13 has been shown to increase tumor antigen uptake and presentation, resulting in enhanced activation of tumor-specific cytotoxic T cells.

The combination of these facts has led to the intriguing possibility that blocking CD13 may amplify the efficacy of MPLA, and combination of the two could generate the "super adjuvant" needed in cancer vaccines. Therefore this project is designed to investigate the combinatorial adjuvant-effect of blocked CD13 and MPLA utilizing the HPV-driven cancer model.

The role of Prostate Specific Membrane Antigen (PSMA) in metastasis

Danica Anukam, Anisha Lewis, Linda Shapiro, Leslie Caromile

Metastasis is the leading cause of prostate cancer (PC) mortality. The metastatic cascade represents a multi-step process that includes the loss of adhesion of primary tumor cells, intravasation into the blood vessels and lymphatics with subsequent extravasation at distant sites, and the formation of new colonies most commonly in the bone which are dependent on integrin-dependent cell adhesion. The current therapy for metastatic spread of PC is chemotherapy in combination with targeted hormonal therapy. However, most PC develops drug resistance with no effective treatment. Prostate Specific Membrane Antigen (PSMA), a type II transmembrane peptidase, is progressively and specifically upregulated in ~80% of tumors during PC progression, where it correlates negatively with prognosis. We have demonstrated that PSMA promotes primary tumor progression in vivo by directly interfering with tumor-suppressive pathways and driving a pro-tumorigenic, anti-apoptotic phenotype. Additionally, we have shown that PSMA stimulates cell surface integrin molecules to forge new linkages with the ECM and other cells, and thus may contribute to metastatic mechanisms in addition to effects on signal transduction.

To assess the effects of PSMA on metastasis, we analyzed anchorage independence, cell proliferation, and tumor progression. Using a human prostate cancer cell line (LNCaP), we deleted PSMA by CRISPR/Cas9 and compared these PSMA knockout (KO) cells to the parental-PSMA expressing cells. Using a soft agar assay to assess the oncogenicity of PSMA, it was shown that the PSMA-expressing cells were able to form larger colonies in an anchorage-independent manner compared to the PSMA KO cells, suggesting that the PSMA-expressing cells were able to grow and divide irrespective of their surrounding environment. Furthermore, PSMA-expressing cells proliferated at a higher rate compared to PSMA KO cells. In xenograft models, it was shown that the PSMA-expressing cells. Lastly, we plan to examine the PSMA effects on metastasis through the usage of a cell-adhesion assay, a transwell migration assay, and flow cytometry to observe apoptosis in both the PSMA-expressing cells and the PSMA KO cells.

Title: Mathematical Modeling of Prostate Specific Membrane Antigen (PSMA) Signaling in Glutamate Addiction and Prostate Cancer Progression

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Prostate cancer ranks second among the major classes of carcinomas in men. In addition, prostate malignancies display great resistance to current therapeutic methods, which is further hampered by a paucity of mechanistic detail underlying prostate cancer progression. In this work, we propose the use of a class of discrete mathematical models, more specifically, a dynamic Boolean network model to understand the regulatory role of glutamate and prostate specific membrane antigen (PSMA) signaling in prostate cancer cells. With this approach, we will construct a metabolomics model that will include key components involved in the signaling cascade for glutamate metabolism and its link to oncogenic pathways, which can then be further used to interrogate regulatory relationships. Thus, the model will focus on the cell fate in response to both extracellular and intracellular glutamate levels and its dependency to prostate cancer progression, in addition to identifying aberrant cellular components. Furthermore, a dynamic Boolean network model that is validated by experimental results can be used as a tool to perform gene knock-out/over-expression simulations, thus offering a guide to generate new hypotheses and neither supplanting valuable experimental methods. Ultimately, the goal of this project is to invoke Boolean network models to gain a mechanistic insight of PSMA signaling cascade in prostate cancer and to further underscore the use of mathematical models to aid in solving biological conundrums.

Ultrastructural organization of the glomerulus and juxtaglomerular complex

Justin Sardi and Mark Terasaki

The glomerulus is a complex structure that has a central role in mammalian kidney function. We are studying its organization by using newly developed methods of serial section electron microscopy. The vascular network within a glomerulus was analyzed from 160 serial 0.5 um sections. This network originates as a single arteriole loop and ramifies during development. It seems that efficient filtration would be best served with a long path length within the network from the afferent to efferent arteriole. How could such a network develop from a single loop? We hypothesized that the ramification involves splitting of arterioles (intussception) rather than sprouting of new branches and fusion, which could lead to the creation of short paths and further, that the development could be deduced from the branching pattern. The branch points and connections were documented; 271 branch points were identified. By writing a Matlab program to do simple network theory analysis, we found that the shortest path from the afferent to efferent arteriole involves 15 branch points. Therefore, the blood does indeed take a relatively long path in going through the glomerulus. However, the branching pattern we found is not obviously the result of intussception, so we were unable to test the hypothesis from the branching pattern. Unexpectedly, we found short segments that form dead ends. These are approximately 3-4 um long; they would not accomodate an 8 um diameter red blood cell and do not seem to be in the process of elongating. There were about 8 dead ends in the analyzed glomerulus. Currently, we are focused on characterizing the juxtaglomerular apparatus region in serial 60 nm sections. This region includes the sodium sensing macula densa, the renin secreting juxtaglomerular cells, and sympathetic innervation.

Epidermal Growth Factor Signaling Regulates Limb Regeneration Responses in Mammals

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In the United States, roughly 185,000 limb amputations occur each year due to severe trauma to the arm or leg. As a result of traumatic limb loss, amputees suffer an impaired quality of life, both mentally and physically. Currently, there are limited resources beyond rehabilitation using prosthetic technology to functionally restore a limb following traumatic limb loss. Animals such as the axolotl (Mexican salamander) can successfully regenerate their limbs but in mammalian species, such as humans and mice, spontaneous limb regeneration is restricted to the digit tip. The mammalian regeneration response is amputation level-specific; injury at the distal-third of the terminal phalanx (phalanx 3 or P3) prompts a spontaneous regeneration response that fully restores functionality as well as adult digit structures in the digit tip after 6-8 weeks, while a more proximal amputation does not elicit a regeneration response. Additionally, amputation at the level of the second phalanx (P2) results in a regeneration - incompetent "healing" response which consists of scar tissue and a nonfunctional bone stump, with little elongation past the site of injury. This non-regenerative, repair response, is consistent with the clinical presentation of traumatic limb loss.

The molecular mechanisms that control level-specific limb regeneration responses in mammals are not well understood. Our previous studies have described that signals from the Epidermal Growth Factor Receptors (EGFR) are vital to limb skeletal progenitor cell proliferation and differentiation. More recently, our studies demonstrate a genetic requirement for EGFR signaling in mediating a complete regeneration response in P3 amputated murine digits. The overall goal of this project is to determine if stimulating EGFR signals can restore a regeneration response in otherwise regeneration-incompetent mouse digits following P2 amputation. For our study, we used a transgenic EGFR gain of function mouse model, in which natural repression of EGFR signaling is removed through conditional genetic loss of the endogenous pan-EGFR family negative regulator Mig6. Importantly, sufficient availability of suitable ligands is required for EGFR gain of function in this model. Digits of Mig6-CKO mice were humanely amputated at the P2 level and collected 8, 11, and 15 days post amputation. Surprisingly, histological analysis revealed a delayed repair response in our EGFR transgenic model compared to controls. This result suggests that availability of endogenous pro-regenerative EGFR family ligands may be limiting in our model; and raises the possibility that exogenous application of select EGFR ligands might be required for a successful P2-level regeneration response. To this end, we characterized the localization of the EGF receptors in order to appropriately select ligands that would potentially drive EGF receptors activation. Through immunohistochemistry, we identified multiple EGF receptors co-localized in the periosteum, cartilage, and bone of the digit. Additionally, we found that certain EGF receptors were activated in the digit stump tissue after

amputation, identifying them as regeneration-regulated signals. This information will be used to identify the appropriate EGFR family ligands that may successfully promote a regeneration response in otherwise regeneration-incompetent limb tissue.

Dephosphorylation and inactivation of the NPR2 guanylyl cyclase is a component of the signaling network by which FGF decreases bone growth.

Leia C Shuhaibar, Jerid W Robinson, Ninna P Shuhaibar, Jeremy R Egbert, Giulia Vigone, Valentina Baena, Melanie C Fisher, Caroline Dealy, Lincoln R Potter, Laurinda A Jaffe.

Cyclic GMP is a positive regulator and fibroblast growth factor (FGF) is a negative regulator of the growth of long bones and vertebrae, but how these regulatory systems interact is incompletely understood. Here we demonstrate that FGF signaling inhibits cGMP production in the growth plate, through dephosphorylation and inhibition of the guanylyl cyclase NPR2. We find that bones are longer in mice in which endogenous NPR2 is replaced with a glutamate-substituted protein (NPR2-7E) that mimics a constitutively phosphorylated enzyme that cannot be inactivated by dephosphorylation. By imaging the growth plates of live tibia from newborn mice expressing an optical sensor for cGMP, we find that wildtype chondrocytes, but not chondrocytes from NPR2-7E/7E mice, show decreased NPR2 guanylyl cyclase activity in response to FGF. These results indicate that a decrease in cGMP resulting from dephosphorylation and inactivation of NPR2 is a component of the signaling network by which FGF decreases bone growth. These studies have the potential to develop new therapeutic strategies to treat skeletal dysplasias, such as achondroplasia (the most common type of dwarfism).

Increasing radiation dose improves immunotherapy outcome and prolongation of tumor dormancy in a subgroup of mice treated for advanced intracerebral melanoma

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Abstract: Previously, we developed a clinically relevant therapy model for advanced intracerebral B16 melanomas in syngeneic mice combining radiation and immunotherapies (Smilowitz et al., 2013). Here, 7 days after B16-F10-luc2 melanoma cells were implanted intracerebrally (D7), syngeneic mice with bioluminescent tumors that had formed (1E105 to 7E106 photons per minute (>1E106, large; <1E106, small) were segregated into large-/small-balanced subgroups. Then, mice received either radiation therapy alone (RT) or radiation therapy plus immunotherapy (RT plus IT) (single injection of mAbPC61 to deplete regulatory T cells followed by multiple injections of irradiated granulocyte macrophage colony stimulating factor transfected B16-F10 cells) (RT plus IT). Radiation dose was varied (15, 18.75 or 22.5 Gy, given on D8), while immunotherapy was provided similarly to all mice. The data support the hypothesis that increasing radiation dose improves the outcome of immunotherapy in a subgroup of mice. The tumors that were greatly delayed in beginning their progressive growth were bioluminescent in vivo- some for many months, indicating prolonged tumor "dormancy," in some cases presaging long-term cures. Mice bearing such tumors had far more likely received radiation plus immunotherapy, rather than RT alone. Radiotherapy is a very important adjunct to immunotherapy; the greater the tumor debulking by RT, the greater should be the benefit to tumor immunotherapy.

Smilowitz, H.M., Sasso, D., Lee, E., Goh, G., Micca, P.L., Dilmanian, F.A. 2013. Therapy model for advanced intracerebral B16 mouse melanoma using radiation therapy combined with immunotherapy. *Cancer Immunology Immunotherapy* 62:1187-1197 pdf

IV Injected Gold Nanoparticles (AuNPs) Access Intracerebral F98 Rat Gliomas More Specifically Than AuNPs Infused Directly Into The Tumor Site By Convection Enhanced Delivery

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ABSTRACT:

It has previously been shown that IV infused Heavy Atom Nanoparticles (HANPs), in this case gold nanoparticles (AuNPs), greatly enhance radiation therapy of advanced subcutaneous tumors (Hainfeld et al., 2004, 2010) as well as orthotopic gliomas in mice (Hainfeld et al., 2013). Local radiation dose enhancements of four-fold are possible with tissue concentrations of 2% by weight AuNPs and produced 50% long-term cures. If this enhancement can be confined to areas within and immediately surrounding the tumor, it may offer a novel means of targeted radiotherapy to limit loss of healthy brain tissue. In this study we compared the biodistribution of AuNPs with respect to tumor cells and tumor edema after either IV infusion or direct infusion into the orthotopic tumor site by convection-enhanced delivery (CED). We have shown that IV infused AuNPs reach a large proportion of the tumor cells, including tumor cells that have migrated far away from the main tumor mass. While directly infused AuNPs have the advantage of being stereo-tactically injected into the tumor site, the bulk of the directly infused AuNPs show little penetration into the main tumor mass and do not localize well to tumor cells that have migrated distant to the primary site. Conclusions: IV infusion shows improved specific localization of HANPs to the tumor compared to direct infusion with CED. Therefore, IV infused HANPs would be expected to enhance radiation therapy of gliomas more specifically and possibly better than CED directly infused HANPs. Further, lower dose radiation therapy to a larger area of the brain after IV HANP treatment would be expected to be more effective than current high dose focused radiation therapy in the absence of HANPs.

Hainfeld, J.F., Slatkin, D. N., Smilowitz, H.M. 2004. The use of gold nanoparticles to enhance radiotherapy in mice. *Physics in Medicine and Biology*, 49: N309 (PMID 15509078). Note: This paper has been selected for inclusion in Institute of Physics (IOP) Select, Institute of Physics, London, England. <u>pdf</u>

Hainfeld, J.F., Dilmanian, F.A., Zhong Z., Slatkin, D.N., Smilowitz, H.M. 2010 Gold nanoparticles enhance radiation therapy of a squamous cell carcinoma growing in mice. *Physics in Medicine and Biology*, 55: 3045-3059 (PMID 20463371). pdf

Hainfeld, J.F., Smilowitz, H.M., O'Connor, M.J., Dilmanian, F.A., Slatkin, D.N. 2012. Gold nanoparticle imaging and radiotherapy of brain tumors in mice. *Nanomedicine London* (Future Medicine).8:1601-1609. <u>pdf</u>

Endothelial Rbfox2 Splicing Factor Regulates the Arterial Response to Platelets and Monocytes Under Low and Disturbed Flow

<u>Patrick A. Murphy</u>, Vincent Butty, Paul L. Boutz, Shahinoor Begum, Christopher B. Burge, Richard O. Hynes

Low and disturbed flow drives the progression of arterial diseases including atherosclerosis and aneurysms. The endothelial response to flow and interactions with recruited platelets and monocytes determines disease progression. Here, we report widespread changes in alternative splicing in the flow-activated murine arterial endothelium *in vivo*. Among the regulated splicing events were Fn-EIIIA and Fn-EIIIB exons, required for the prevention of intimal hemorrhage under low flow. These, and other skipped exons were regulated by platelets and macrophages recruited to the arterial endothelium under low and disturbed flow. Motifs for the Rbfox-family are enriched adjacent to the regulated exons and endothelial deletion of Rbfox2, the only family member expressed in arterial endothelium, suppresses a large portion of the RNA splicing and transcriptional changes induced by low flow. Our data reveal an alternative splicing program activated in the endothelium by platelet and macrophage recruitment and demonstrate its relevance in flow-driven vascular inflammation.

Novel Heavy Atom Nanoparticles to Enhance Radiation Therapy in Primary and Metastatic Brain Tumors

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Abstract: It was previously shown that IV infused heavy atom nanoparticles (HANPs), in this case gold nanoparticles (AuNPs), greatly enhance radiation therapy of advanced subcutaneous tumors (Hainfeld et al., 2004, 2010) as well as orthotopic gliomas in mice (Hainfeld et al., 2013). Local radiation dose enhancements of 4-fold are possible with tissue concentrations of 2% by weight AuNPs and produced 50% long-term cures. Although AuNPs are not toxic when administered at such high levels, they have some drawbacks that may limit their potential for clinical translation including poor clearance, high cost and skin coloration. Here we have begun to study a novel iodine based nanoparticle (INP) developed by Nanoprobes, Inc. for primary and metastatic brain tumor therapy. We describe initial experiments to study the ability of the INPs to enhance radiation therapy of an orthotopically transplanted human glioma growing in the brains of athymic nude mice and correlate efficacy with tumor distribution and loading of INPs and AuNPs. After IV injection AuNPs and INPs are able to localize to advanced U87 tumors: A) Tumor Uptake (Micro CT): Injection of INP at ~3 g/kg resulted in the uptake of ~0.6% iodine in the U87 tumors (measured by MicroCT) which should give an overall dose enhancement of ~2-fold after x-irradiation (Cho et al., 2005). B) Tumor Targeting (Fluorescence and Light Microscopy): Injection of Luc2 and Cherry Red expressing U87 cells into athymic nude mice resulted in the development of advanced gliomas. Twenty-four hours after the IV injection of ~1g/kg AuNP the mice were fixed/perfused and frozen sections of the brains containing tumor were both gold enhanced and stained for markers of edema and endothelial cells. Gold nanoparticles were found localized largely to the outer 40% of the spherical tumor. An enhanced amount of vasculature in the tumor region provides a mechanism for nanoparticle entry into the tumor site. C. Efficacy (IVIS and Survival): Forty mice were implanted with U87 cells expressing RedFLUC. When tumors were progressing and had reached an advanced stage, the mice were divided into three groups of approximately equivalently sized tumors. One group was untreated (control). The second group received radiation-only (single fraction, 30 Gy). The third group received INP (1.75 g/kg) + 30 Gy radiation. All of the mice are being followed by IVIS and body weight. Mice that receive INP and radiation therapy show continual weight gain and reduction in tumor size in comparison to untreated, control mice. More time is needed to assess the efficacy of INP therapy. Conclusion: IV injected INPs load the outer 40% of advanced U87 gliomas to a level that should provide ~2-fold x-ray irradiation dose enhancement to the outer rim of the tumor. An experiment to assess efficacy is In Progress.

Hainfeld, J.F., Slatkin, D. N., Smilowitz, H.M. 2004. The use of gold nanoparticles to enhance radiotherapy in mice. *Physics in Medicine and Biology*, 49: N309 (PMID 15509078). Note: This paper has been selected for inclusion in Institute of Physics (IOP) Select, Institute of Physics, London, England. <u>pdf</u>

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KEYNOTE SPEAKER

"Biography of a Nanoparticle"



James F. Hainfeld, PhD

President, Founder, and Chief Research Scientist Nanoprobes, Inc. Yaphank, NY

Dr. Hainfeld is a senior scientist, his world bridging biology, physics and chemistry. One of the original fathers of the nanoparticle, he engineered some of the very earliest under an electron microscope at Brookhaven National Laboratory in the 1970s. After founding his own laboratory at Nanoprobes in 1990, Dr. Hainfeld has pursued his passion: seeking cures for cancer and other diseases, using specially engineered nanoparticles.

He is the winner of the 2011 Röntgen Prize from the British Institute of Radiology, for special merit and contributing materially to the advancement of the science and practice of radiotherapy, radiobiology, and physics.