**Courtney Betts**  
**Schedin Lab**  
**Cell, Developmental and Cancer Biology Department**

**Collagen-dendritic cell interactions may contribute to parity-induced breast cancer protection.**

Courtney Betts, Kirk Hansen, Virginia Borges, Pepper Schedin

**Abstract:** Epidemiological studies have identified early age pregnancy as protective against a woman’s life-long breast cancer risk. Previous studies have demonstrated that changes in the mammary epithelium likely contribute to this protection. Since stroma has been show to regulate cell behavior, there may be stromal components of this protection. Using in vitro and in vivo models, our lab has shown that extracellular matrix (ECM) isolated from the mammary glands of parous rodents is sufficient to reduce tumor growth and the invasive phenotype of tumor cells. ECM from the parous mammary gland contains mechanically soft and randomly organized collagen fibers. Tumor cells exposed to a 3D recapitulation of this ‘parous’ collagen display a dormant phenotype, and up-regulate genes involved in type I IFN signaling, which is linked to dendritic cell activation. **These studies raise the novel hypothesis that parous collagen organization may activate dendritic cells resulting in tumor suppression.** My recent studies have found that ‘parous’ collagen induces dendritic cell activation and production of anti-tumor cytokines (IL-12, IFNγ, TNFα) to a greater extent than ‘nulliparous’ collagen. These findings suggest that physiologically normal parity-induced collagen structural changes may enhance dendritic cell activities anticipated to be anti-tumor and contribute to pregnancy-induced breast cancer protection.

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**Amy Bittel**  
**Gibbs Lab**  
**OHSU Center for Spatial Systems Biomedicine**

**Design and Development of BODIPY-based photoswitchable fluorophores for Multi-Spectral Super Resolution Microscopy (MSSRM)**

Amy M. Bittel, Andrew K. Nickerson, Li-Jung Lin, Xiaolin Nan, Summer L. Gibbs

**Abstract:** Super resolution microscopy (SRM) has overcome the historic spatial resolution limit of light microscopy, enabling fluorescence visualization of cellular structures and multi-protein complexes at the nanometer scale. Using single-molecule localization microscopy, the precise location of a stochastically activated population of photoswitchable fluorophores is determined during the collection of many images to form a single image with resolution of ~10-20 nm, an order of magnitude improvement over conventional microscopy. However, the spectral resolution of current SRM techniques are limited by existing fluorophores with only up to four colors imaged simultaneously, limiting the number of intracellular components that can be studied in a single sample. In the current work, a library of novel BODIPY-based fluorophores was synthesized using a solid phase synthetic platform with the goal of creating a set of photoswitchable fluorophores that can be excited by 5 distinct laser lines but emit throughout the spectral range (450-850 nm) enabling multispectral super resolution microscopy (MSSRM).
Shanta Boddapati, Ph.D.
Gray Lab
OHSU Center for Spatial Systems Biomedicine

Nanoimmunological insights into PI3k/mTOR pathway inhibition uncovers heterogenous Akt isoform activation associated with PTEN mutation status in breast cancer cell lines

Shanta Boddapati¹, Wallace Thompson¹, Nora Bayani², Suzi Fei¹, Nupur Pande¹, Sunjong Kwon¹, Koei Chin¹, Kristina Iljin³, Nick Wang¹, Jim Korkola¹ and Joe Gray¹

Abstract: Breast cancers are characterized by genomic aberrations that alter signaling through crucial pathways like the PI3k/Akt/mTOR pathway. These include frequent activating mutations in the lipid kinase PIK3CA and inactivating mutations in the phosphatase PTEN that negatively regulates this pathway. Phosphorylation and activation of a crucial member of this signaling cascade – the Akt kinase at Serine 473 and Threonine 308, results in increased breast cancer cell growth and survival. Interestingly, three highly homologous isoforms of Akt are expressed in breast cancers with non-redundant downstream targets and functions. Several inhibitors have been developed to target various kinases involved in this pathway. However, their effects on Akt isoforms have not been analyzed. We have used a novel nanoimmunoassay to characterize phosphorylation changes in Akt isoforms and show that PTEN null cell lines do not dephosphorylate Akt2 when treated with a PI3k/mTOR inhibitor unlike PIK3CA mutant cell lines. Additional mtor inhibition abolishes this effect suggesting that mtor signaling differentially phosphorylates the Akt isoforms. Combined drug and siRNA studies will be used to further study the mechanism of PTEN association with the differential phosphorylation of Akt isoforms. These results have strong translational implications for effective personalized combination therapy treatment in breast cancer.

Marilynn Chow
Tyner Lab
Cell, Developmental and Cancer Biology Department
*ePoster Presenter*

ROR1 regulates AKT phosphorylation through functional interactions with the MTORC2 complex in T(1;19) B-cell acute

Chow, Marilynn; Tyner, Jeffrey, W.

Abstract: The mammalian target of rapamycin complex 2 (mTORC2) activates the serine/threonine protein kinase, AKT, through phosphorylation of the Serine-473 residue. In response to the small molecule SRC-family inhibitor Dasatinib, AKT phosphorylation is reduced in t(1;19)-ALL cells, which are arrested at intermediate stages of B-lineage maturation. t(1;19)-ALL cells are also dependent on the highly expressed pseudokinase, receptor tyrosine kinase-like orphan receptor 1 (ROR1). Treatment of t(1;19)-ALL cells with Dasatinib leads to increased ROR1 expression and a partial rescue of AKT phosphorylation, suggesting a ROR1-dependent compensatory mechanism. However, the mechanism allowing ROR1 activate the AKT cascade remains unclear. We analyzed endogenous ROR1-mTORC2 interactions with co-immunoprecipitation experiments using whole cell extracts from a cell line that possesses a t(1;19) translocation. Preliminary data suggests that ROR1 interacts with Rictor, which is critical for mTORC2 catalytic activity. Furthermore, silencing ROR1 expression with siRNA led to reduced Rictor expression compared to cells treated with nonspecific siRNA. These data suggest that ROR1 positively regulates AKT activation through interactions with mTORC2, and ROR1 may serve as a previously unrecognized scaffold for the assembly of mTOR signaling complexes. This work will help to elucidate unknown mechanistic details underlying the role of ROR1 in AKT signaling and leukemic cell survival.
Paige Davies, Ph.D.
Wong Lab
Cell, Developmental and Cancer Biology Department

Bmi1 intestinal stem cells give rise to differentiated Paneth cells of the crypt niche
Paige S. Davies, Kelley S. Yan, Calvin J. Kuo, and Melissa H. Wong

Abstract: The field of intestinal stem cell research has made great strides in the last decade with the identification of numerous stem/progenitor cell populations; however, a fundamental understanding of the diverse regulation of distinct stem cell populations is lacking. It is evident that some niche cells are rapidly cycling (Lgr5+), whereas others are more quiescent (Bmi1+); yet it remains unclear which factors regulate the quiescent intestinal stem cell to become activated. The intestinal epithelium has a varied response to injury that plays on the dynamic relationship between the Lgr5+ and Bmi1+ intestinal stem cell populations. In response to irradiation damage, the Lgr5+ cells are lost and Bmi1+ intestinal stem cells provide the regenerative force. Although the Bmi1+ intestinal stem cell is primarily slow cycling, this ability to drive epithelial renewal upon activation, suggests a tight regulation of proliferative capacity. Our data from both Bmi1-GFP reporter mice and Bmi1-Cre lineage tracing experiments demonstrate that Bmi1+ cells can directly give rise to a differentiated cell population within the crypt niche, Paneth cells, highlighting a potential mechanism for efficient repair in the face of intestinal epithelial damage.

Catherine Drerup, Ph.D.
Nechiporuk Lab
Cell, Developmental and Cancer Biology Department

Actr10 regulates retrograde mitochondrial transport
Catherine M. Drerup, Sarah Lusk, Alex Nechiporuk

Abstract: The formation and maintenance of neural circuits requires the active transport of proteins and organelles in neural processes. Retrograde transport (towards the cell body) is accomplished by one motor complex, cytoplasmic dynein. The diversity of cargos moved by dynein and the regulation of their deposition implies the existence of cargo-specific modulators of retrograde movement. One cargo whose retrograde movement is essential for axon formation and maintenance is mitochondria. These organelles are actively transported to sites with high metabolic demands and synaptic activity as they supply energy and maintain calcium homeostasis. How these organelles attach to dynein for retrograde transport is unknown. In a genetic screen, we isolated a zebrafish mutant with mitochondrial accumulations in axon terminals due to defects in retrograde movement. Subsequently, we identified the causative mutation as a T to G change in the start codon of the gene encoding Actr10, a member of the dynein-interacting complex dynactin. Though this protein is ideally situated to serve as a cargo adaptor, a role for Actr10 in dynein-mediated cargo transport has not been reported. Using a combination of in vivo biochemistry and live imaging, future work will define the mechanistic role of Actr10 in mitochondrial retrograde transport.
David Edwards  
Tyner Lab  
Cell, Developmental and Cancer Biology Department

**Characterizing the Effects of CSF1R Inhibition in Acute Myeloid Leukemia with RUNX1 Abnormalities**

David K Edwards V, Matthew Siegel, Jill Peters, Anupriya Agarwal, Alyssa Carey, Melissa L Abel, Shannon K McWeeney, Jeffrey W Tyner

**Abstract:** Acute myeloid leukemia (AML) is the deadliest hematological malignancy, resulting in 10,320 cancer deaths in the United States in 2013. While targeted therapy has increasingly become an effective strategy in cancer treatment, few targeted therapies exist for AML. One recurrently altered gene, Runt-related transcription factor 1 (RUNX1), is found to be abnormal in ~20% of AML cases. RUNX1 abnormalities are loss-of-function, making them poor drug targets, so it is important to consider targeting downstream effectors that are differentially regulated by abnormal RUNX1. Previously, we have developed functional screening approaches in which patient cells are exposed to siRNA kinome panels or small molecule inhibitors and cell viability is measured. Using these techniques in hundreds of patient samples, we observed that samples with RUNX1 aberrations are uniquely sensitive to inhibition/silencing of CSF1R, a downstream target of RUNX1. We are conducting experiments to determine if RUNX1 knockout in AML cell lines recapitulates the sensitivity to CSF1R inhibition observed in patient samples, and to understand the mechanism behind RUNX1 regulation of the CSF1R pathway in AML. The results of this research have direct applications in the clinic, where eventually CSF1R inhibitors could be used a novel targeted therapy in AML patients with RUNX1 abnormalities.

Amanda Esch, Ph.D.  
Spellman Lab  
OHSU Center for Spatial Systems Biomedicine

**Quantitating heterogeneous drug response in single cells by high-throughput microscopy and mass cytometry**

Amanda Esch, Katie Johnson-Camacho, Juha Rantala, Nicholas Wang, Tyler Risom, Paul Spellman, Joe Gray

**Abstract:** Breast cancer is the most common cancer worldwide in women, constituting more than 25% of new cases diagnosed. Current strategies for treatment of breast cancer include use of targeted agents and chemotherapeutics; however, many patients develop resistance to these compounds and eventually succumb to metastatic disease. One significant impediment to the effective treatment of breast cancer is intratumoral heterogeneity. Individual cells within a tumor may respond differently to therapeutics, leading to expansion of resistant subpopulations with increased proliferative and metastatic capabilities. In order to reduce the risk of disease recurrence and metastasis-related death, it is necessary to identify cellular phenotypes of resistant cells and target these therapeutically. We are utilizing high-throughput microscopy (scanR) and mass cytometry (CyTOF) to define subpopulations in heterogeneous breast cancer cell lines and to assess responses of individual cells to drug treatments. These technologies enable analysis of phenotypic properties of cells and tissues as well as quantitation of pharmacodynamic markers of proliferation, apoptosis, senescence, cell cycle phase, and cell lineage. Using these approaches, we are working to identify drug combinations that can be used therapeutically to effectively eliminate all subpopulations within a tumor.
Charlie Gast
Wong Lab
Cell, Developmental and Cancer Biology Department

Development of a metastatic mammary cancer model that mimics patient disease

Gast C.E., Shaw A., Ruffell B., Coussens L.M., Wong M.H.

Abstract: Breast cancer represents the second highest mortality for cancers in women. Despite advances in early detection, breast cancer deaths remain high due to metastatic disease. Lumpectomy or mastectomy followed by adjuvant chemotherapy is a common treatment strategy for patients. Recently, immune modulating compounds have shown promise in treating mammary cancer metastases in mice without primary tumor resection. Because breast cancer patients undergo surgical resection of their primary tumor prior to metastatic disease treatment, development of mouse models that mimics this course is critical for identifying novel immunotherapies to treat breast cancer.

To establish an adjuvant mouse model, tumor cells from transgenic MMTV-PyMT mice were dissociated then injected into fat pads of wild-type mice. Tumors were surgically excised followed by temporal analyses of lung metastases conducted by live-imaging and tissue analyses. Despite lack of primary tumor regrowth, metastatic growth in the lungs were identified in 75% of the mice, demonstrating that tumor cell seeding of the lung occurs during primary growth phase of the tumor and mimics the course of human breast cancer progression. Our mouse model for surgical resection and metastatic tumor progression provides a platform to evaluate novel adjuvant therapies and the biology underlying metastatic cell growth.

Qiuchen Guo
Schedin Lab
Cell, Developmental and Cancer Biology Department

Pro-tumorigenic collagen deposition during mammary gland involution

Qiuchen Guo, Pepper Schedin

Abstract: Postpartum mammary gland involution is tumor promotional in rodent models and is suggested to account for the poor prognosis of breast cancers diagnosed in recently pregnant women. One potential contributor is collagen remodeling. Increased collagen deposition and straight fiber formation during involution are implicated in breast cancer metastasis. Fibroblasts and macrophages are important cells types involved in collagen remodeling. Murine mammary PDGFRα+ fibroblast density increases ~4-fold during involution and these cells have increased collagen I, III and lysyl oxidase expression compared to nulliparous, suggesting that fibroblasts are specifically recruited to and activated within the involuting microenvironment, contributing to involution-specific collagen remodeling. Macrophages can impact collagen organization and are known to be essential during postpartum gland involution. Using flow cytometry, we find two distinct populations of macrophages in the mammary gland, F4/80 high (++) and F4/80 medium (+). Cytokine profiles differ between these two populations and are further modulated by reproductive stage. F4/80+ cells express high IL12 in nulliparous, whereas F4/80++ cells have increased IL10 expression specifically during involution. These data are consistent with immune suppression characteristic of involution. Future studies using in vitro model to investigate macrophages and fibroblasts cross-talk may provide insights into therapeutic targets of postpartum breast cancer.
Andrew Gunderson, Ph.D.
Coussens Lab
Cell, Developmental and Cancer Biology Department

BTK antagonism diminishes pancreatic cancer growth via dual regulation of FcRγ and B regulatory cells

Andrew J. Gunderson

Abstract Using a transgenic mouse model of squamous carcinogenesis, we previously reported that B cells foster the progression of solid tumors by depositing circulating immune complexes into neoplastic parenchyma thereby activating FcRγ regulated pro-tumoral myeloid phenotypes that diminish the chemotaxis of CD8⁺ T cells. Accordingly, treating SCC-bearing mice with a B cell-depleting monoclonal antibody (αCD20 mAb) in combination with chemotherapy inhibits tumor growth; efficacy dependent on the presence of CD8⁺ T cells. Human pancreatic ductal adenocarcinomas (PDA) also exhibit significant B cell residency and IgG deposition suggesting this cancer might also be susceptible to B cell-ablation as a therapeutic strategy. Orthotopic PDA tumor growth in mice genetically deficient in B cells or FcRγ was attenuated, however, αCD20 mAb monotherapy or with gemcitabine failed to reduce tumor growth. IL-10 expressing B regulatory cells were resistant to αCD20 depletion and sufficient to restore PDA growth. Likewise, neutralizing IL-10R prior to gemcitabine therapy was effective at limiting tumorigenesis, again dependent on CD8⁺ T cell immunity. In combination with gemcitabine, treatment with the small molecule BTK inhibitor, Ibrutinib, limited tumor burden by dual inhibition of BCR and FcRγ signaling, thereby simultaneously negating both B cell and myeloid cell mediated mechanisms of PDA development.

Saima Hassain, M.D., Ph.D., FRCSC
Gray Lab
OHSU Center for Spatial Systems Biomedicine

Preclinical modeling of therapeutic response to PARP inhibition in breast cancer

Saima Hassan, Laura Heiser, Joe Gray

Abstract: Predicting therapeutic efficacy in clinical trials from the laboratory remains a challenging process. One approach to overcome this problem is to better select a patient population a priori that may most benefit from a specific therapeutic agent. PARP inhibitors target DNA damage response pathways and have been shown to be more effective in a more aggressive form of breast cancer, triple-negative disease. In the I-SPY 2 TRIAL, patients treated with the combination of a PARP inhibitor, carboplatin, and paclitaxel prior to surgery, demonstrated a higher estimated rate of pathologic complete response, in comparison to patients treated with paclitaxel alone. Our aim is to identify genomic predictors of response in in the laboratory using a panel of breast cancer cell lines that will be correlated with genomic predictors from breast cancer patients treated with the same PARP inhibitor. Using live-cell imaging, we have identified therapeutic response with the PARP combination upon cellular proliferation. Maintaining the concentrations of the therapeutic agents within a physiologic range, we modulated the concentrations in order to disentangle the contribution of each agent to the combination. We will then test the PARP inhibitor upon various functional endpoints in order to better understand its impact upon cellular function.
Laura Heiser, Ph.D.
Gray Lab
OHSU Center for Spatial Systems Biomedicine

A community-based effort to identify robust signaling network inference algorithms

Laura Heiser, Steven Hill, Thomas Cokelaer, Michael Unger, Thea Norman, Mike Kellen, Bruce Hoff, Jay Hodgson, Nicole Nesser, Gordon Mills, Joe Gray, Stephen Friend, Paul Spellman, Heinz Koeppl, Julio Saez-Rodriguez, Sach Mukherjee, and Gustavo Stolovitzky

Abstract: Signaling networks play a key role in the regulation of cell processes such as growth and apoptosis, and their dysregulation is central to many diseases, including cancer. Although there is a wealth of literature describing canonical cell signaling networks, relatively little is known about exactly how these networks operate in different cancer cells. We hypothesize that causal signaling links and system dynamics are dependent on both lineage and (epi)genetic background. Identification of the signaling networks requires computational approaches that can robustly predict protein phosphorylation dynamics from data acquired in a specific biological context. To that end, we have participated in the DREAM Project, a community-based effort to rigorously assess novel algorithms using standardized metrics and blinded gold standards. In the DREAM8 Challenge, an extensive training dataset comprised of phospho-proteomic time-courses from four breast cancer cell lines, acquired under eight different ligand stimuli and under inhibitor perturbations, was provided to the scientific community. Overall, this community-based approach was able to rapidly and robustly identify several top-performing algorithms for network inference, and serves as a model for community-based collaboration.

Thomas Jacob, Ph.D.
Vu Lab
OHSU Center for Spatial Systems Biomedicine

Single cell supersensitive phosphoprotein profiling quantum dot assay platform for heterogeneous drug response analysis

Thomas Jacob, Anupriya Agarwal, Thomas O’Hare, Damien Ramunno-Johnson, Brian J. Druker, Tania Q. Vu

Abstract: Quantification of cellular phosphoprotein is critical for understanding drug response sensitivity and PP target effects. We present a drug profiling platform that images single, intensely bright phosphoportein antibody-bound fluorescent quantum dot (QD) nanoparticles. The single cell quantum dot phosphoassay employs an optimized antibody-QD probe panel, a customized multi-well assay chamber that accommodates situations of limited cell number, and software for automated 3D multichannel image acquisition and nanoparticle detection analysis that provide rapid, high-throughput workflow. The sensitivity of our PP-QD platform enables quantification of single activated PP complexes in single cells, thereby exceeding techniques (Western blot, FACS) currently limited by sensitivity or ensemble detection (PP levels in large cell populations). The medical value of this technique is shown by identification of single drug-resistant CD34 stem cells in leukemia patients.
Abstract: Juvenile myelomonocytic leukemia (JMML) is a rare childhood myeloproliferative disorder with high recurrence and mortality. The only cure is allogeneic hematopoietic stem cell transplant. White blood cells from a patient with recurrent JMML were found to be sensitive to several tyrosine kinase inhibitors, including dasatinib. This patient’s sample had reduced viability when treated with an siRNA against tyrosine non-kinase 2 (TNK2), a target of dasatinib, and a possible regulator of signaling downstream of RAS. The patient was placed on dasatinib and achieved marked improvement.

This patient had a mutation in the tyrosine phosphatase SHP2, which acts on pathways including JAK-STAT, PI3K-AKT, and RAS-MAPK with an overall positive effect on proliferation. Mutation of SHP2 in the same region as our patient’s mutation leads to increased proliferation and cell survival and is linked to dysregulation of the RAS signaling pathway. These results suggest dasatinib as a possible therapy for JMML patients and that dysregulation of cellular signaling pathways by these mutations results in a TNK2-dependent sensitivity to dasatinib. Experiments are currently being conducted to verify dasatinib sensitivity in PTPN11 mutant cell lines and to clarify the mechanistic interactions between PTPN11 and TNK2, which will lead to improved therapeutic options for JMML patients.

Abstract: With the prevalence of different color tissue stains and fluorophores used in the life sciences, consideration of the viewer’s ability to discriminate color variation becomes appropriate. This presentation was prepared to alert the microscopy community of the prevalence of color vision deficiency within our audience and to suggest the use of readily available tools to simulate how a micrograph is perceived by affected individuals. We further propose a color scheme which allows striking contrast to both color sighted and color blind individuals. Color blindness is an inherited condition known to affect up to 8% males and 0.4% of females, with geographic variability. The most common forms, protonopia and deuteranopia, are caused by an X-linked recessive allele that affects the ability to perceive the colors red and green due to an absence of the associated photoreceptors [1,2]. For both forms of color blindness, both red and green are perceived as yellow and therefore cannot be distinguished. A powerful tool is found within FIJI (a version of ImageJ) available as a free download (FIJI.sc). FIJI may be used to simulate the perception of the full spectrum of colors and allows one to simulate the perception of any color image by individuals affected with either protonopia or deuteranopia (FIJI/Image/Color/Simulate Color Blindness). Importantly, magenta is perceived as a hue of blue by color blind individuals; therefore Magenta/Green images are perceived as Blue/Yellow. FIJI allows one-button conversion of a Red/Green image to Magenta/Green (FIJI/Image/Color/Convert Red to Magenta). Simulation of color blindness may also be accomplished (with limitations) using Adobe Photoshop/View/Proof Setup/Color Blindness.
Jim Korkola, Ph.D.
Gray Lab
OHSU Center for Spatial Systems Biomedicine

The Library of Integrated Network-Based Cellular Signatures (LINCS) Data and Signature Generation Center (DSGC) at OHSU

James Korkola¹, Laura Heiser¹, Paul Spellman¹, Adam Margolin¹, Mark Labarge², Gordon Mills³, and Joe W. Gray¹

Abstract: The LINCS program aims “to create a network-based understanding of biology by cataloging changes in gene expression and other cellular processes that occur when cells are exposed to a variety of perturbations, and by using computational tools to integrate this diverse information into a comprehensive view of normal and disease states that can be applied for the development of new biomarkers and therapeutics.” The OHSU LINCS-DSGC will analyze cancer and normal cells grown on microenvironment microarrays (MEMA) consisting of more than 2500 unique combinatorial microenvironments (ME) to generate data for public release. The primary data generated will be images and intensity values of cells on MEMA stained for phenotypic markers (proliferation, apoptosis, differentiation state, etc.). Significant ME will be graduated to validation and elastic modulus studies at LBNL. Validated ME will be submitted for Luminex 1000 expression profiling (in collaboration with the Broad LINCS center) and phosphoproteome profiling using reverse phase protein arrays (RPPA, performed at MD Anderson). All resulting data will be fed into the data analysis core at OHSU for development of networks and signatures of response under different ME conditions. This LINCS-DSGC is expected to generate over 12 million data points over the next 6 years.

Sushil Kumar, Ph.D.
Coussens Lab
Cell, Developmental and Cancer Biology Department

Improving Chemotherapy by Targeting Macrophage Function in Malignant Mesothelioma

Sushil Kumar¹, Collin M. Blakely², Raphael Bueno⁸, David J. Sugarbaker³, David M. Jablons⁴, V. Courtney Broaddus⁸, and Lisa M. Coussens¹

Abstract: Malignant mesotheliomas (MM) are markedly resistant to standard chemotherapy (CTX). Recent addition of the folate antagonist pemetrexed to platinum-based CTX has provided a modest 3-month survival benefit. In investigating mechanisms regulating chemoresistance in MM, we found that human MMs are highly infiltrated by macrophages, representing ~40% of total cellularity. Using an experimental murine model of MM, we revealed that response to CTX is significantly improved by blockade of colony stimulating factor receptor (CSF1R) leading to macrophage depletion. Using an ex vivo 3D spheroid model to investigate molecular mechanisms regulating this response, we found that macrophages increase viability of MM spheroids stressed by CTX in part by activation of epidermal growth factor receptor (EGF). Interestingly, treatment of MM spheroids with erlotinib, an EGFR inhibitor, sensitized MM spheroids to CTX. In vivo, in addition to macrophage-derived EGF, macrophages express high levels of PD-L1 thereby impacting CD8+ T cells responses in tumors. Together, our data indicates that macrophages regulate two important axises in MM, EGF-regulated survival programming and the PD-1/PD-L1 pathways regulating CD8+ T cells. We are leveraging these findings to conduct preclinical trials targeting macrophages and these axises to improve response to CTX, results of which will be rapidly translated to the clinic.
**Quantitative co-imaging of mRNAs and protein in Her2-positive breast cancer single cells treated with Lapatinib, a HER-family tyrosine kinase inhibitor**

Sunjong Kwon¹, Michel Nederlof², Patrina Pellett³, Aurelie Snyder⁴, Koei Chin¹, Joe W. Gray¹

**Abstract:** Both intrinsic and extrinsic micro-environmental factors influence pathway function in individual cancer cells, which results in establishing tumor heterogeneity. We have been developing imaging methods to allow simultaneous quantification of RNAs and proteins at the single cell level. First, we established single molecule fluorescent in situ hybridization (smFISH) technology to quantify individual transcripts. Her2 mRNA particle counts are closely related to DNA copy number data in a variety of breast cancer cells. The unexpected nuclear Her2 mRNA aggregates are resolved using super-resolution structural illumination microscopy (SR-SIM). Next, we established “immuno-smFISH,” combining immunocytochemistry and smFISH for the simultaneous co-imaging of protein and RNA, and applied it to time-lapse analyses of Her2 mRNA expression and phosphoAkt protein levels in Her2-positive breast cancer single cells treated with the HER-family tyrosine kinase inhibitor Lapatinib. Nuclear morphometries are also analyzed by measuring the size, intensity, aspect ratio, perimeter, roundness, and circularity of DAPI-stained nuclei, whose differences might cause expression level changes. Our imaging methods provide information about the association between transcription level, cellular localization, and protein expression in individual cells, and would be applied to pinpoint target cancer cells of aberrant signaling and subsequent end-point gene expression in human tumor biopsy samples and xenograft tissues.

**Spatiotemporal live imaging of HER2 receptors and spatial distribution of AKT in breast cancer using quantum dots**

Lam, Wai Yan¹; Ramunno-Johnson, Damien¹²; Bowcock, Alec¹; Chin, Koei¹²; Gray, Joe¹²; Bruchez, Marcel¹⁴; Vu, Tania¹²

**Abstract:** Spatiotemporal coordination of receptors with downstream effectors serves as a fundamental means by which cells transduce signals. HER2 and Akt are PI3K proteins dysregulated in breast cancer. How activated HER2 traffic and interact with downstream effectors to produce signaling is not understood, though it has been suggested that HER2 recycling, and its location relative to downstream effectors is key for signal propagation. Moreover, little is known about the relationship between upstream receptor behavior and downstream effects like pAkt localization. We use a sensitive anti-HER2 affibody-QD probe to observe dynamic trafficking of single receptor complexes in live SKBR3 cells treated with heregulin(HRG), and quantitatively localize single pAkt-QD complexes using customized image-analysis algorithms relative to membrane and nucleus. We find HER2 receptor present on the membrane that localize intracellularly following longer HRG treatment. We find pAkt complexes shift from a predominantly cytoplasmic to membranous distribution 5mins following pulsed stimulation. Such studies tracking HER2 and pAkt allow us to unravel the discrete downstream effects that ensemble measurements overlook. Our capability to follow receptor internalization will reveal HER2 trafficking behavior. This along with the ability to look at downstream effects like pAkt localization will inform our understanding of the spatiotemporal organization of PI3K signaling.
**Abstract:** The most commonly used tools for interrogating living systems are on the micro-scale with dimensions down to one micron. These tools include micropipettes used for patch clamp work and metallic electrical probes. Nanotechnology has a lot to offer in regards to reducing the dimensions of probes and modifying their properties using a host of different materials other than the typical fused silica micropipette and capillary tubes. Recent advancements in nano-fabrication have led to the birth of a new era of cellular perturbation where physical dimensions of active probes can approach one nm. Nano-scale materials had been introduced to living systems before this work, however, the materials were typically solution-borne and injected either *in vitro* or *in vivo*. We report the use of aluminum oxide nanowires supported *via* vertically aligned carbon nanotube scaffolds for delivery of various biological effectors and stimulation of DC 2.4 immune cells. This platform holds promise for cellular manipulation with little impact on cell viability with high throughput analysis of a particular cell population in response to a selected perturbation.

**Abstract:** Cell-intrinsic and extrinsic signals impact tumor cell phenotypes as well as tumor growth, metastasis, and response to therapy. Standard 2D tissue culture has increased our understanding of how cell-intrinsic signals impact tumor biology; however, it has become evident that more complex models that incorporate cell-extrinsic factors are needed. Through a collaboration with Organovo, Inc, we have obtained a 3D bioprinter that is capable of printing discrete structures of multiple cell types in specific patterns. This technology is advantageous over standard 3D culture because it allows for reproducible printing of cellular structures that recapitulate the spatial architecture seen in human tumors. In addition, this model is advantageous over mouse models, as it utilizes human cells, including immune cells, and significantly reduces time and cost to endpoints. Finally, since individual patient tumors can be printed and treated to test therapeutic efficacy, 3D bioprinted tissue may provide a unique platform for personalized medicine. We are currently using this technology to print both breast and pancreatic tumor tissue to interrogate the complex interactions between tumor and stromal cell types and to test the therapeutic efficacy of standard and novel therapies.
Development of a urine based bladder cancer diagnostic using next generation DNA sequencing

Trevor Levin, Jim Korkola, Jason Tee, Joshua Ness, Christopher Amling, Joe Gray, Theresa Koppie

Abstract: Each year in the U.S. nearly 75,000 new cases of bladder cancer are diagnosed with over half a million people living with bladder cancer. For the majority of patients, bladder sparing surgery is a mainstay therapy but risk of disease recurrence is high with over 70% of patients experiencing disease recurrence. As a result, invasive surveillance is recommended by trans-urethral cystoscopy every 3-4 months for years following diagnosis. Trans-urethral scoping is performed by a urologic surgeon with consultation by pathologists making bladder cancer one of the most costly and burdensome cancers to survey over a patient’s lifetime. Towards development of a minimally invasive recurrence diagnostic, we have optimized procedures for extraction of DNA from urine of patients with bladder cancer and non-cancer controls. A targeted DNA sequencing panel was developed to encompass 1,000 loci containing diverse causative genomic lesions in bladder cancer. To date, 25 cancer patients and normal controls have been analyzed. We find that in all cancer patients we are able to detect shared mutations between their primary tumors and DNA from urine collected prior to surgery. Excitingly, recent analysis of longitudinal urine samples suggests that persistence of these markers in urine following surgery may predict future recurrence.

Leukocyte Involvement in the Initiation and Maintenance of Pancreatitis

Shannon M. Liudahl, Andrew J. Gunderson, Christopher J. Chan, and Lisa M. Coussens

Abstract: Pancreatic ductal adenocarcinoma (PDAC) has a low 5-year survival rate, largely due to late detection. Chronic pancreatitis is a risk factor for PDAC; however, the molecular and cellular mechanism(s) underlying this risk remain poorly defined. Since unresolved chronic inflammation, such as chronic pancreatitis, can promote neoplastic progression, we sought to identify critical leukocyte populations or leukocyte-regulated programs involved in potentiating PDAC development. We have previously reported that B lymphocytes foster chronic inflammation that potentiates malignant disease in the skin by activating pro-tumorigenic programs in infiltrating myeloid cells. We have also demonstrated that human PDACs exhibit significant B cell and myeloid infiltration, and B cell-deficient (JH−/−) mice have significantly growth-impaired tumors following orthotopic implant. Therefore, we hypothesize that B cells may also play a role in the initiation or maintenance of pancreatitis. Thus, we are evaluating leukocyte complexity during initiation, maintenance and resolution phases of acute and chronic pancreatitis using immune-competent murine models. Results from these studies are revealing the temporal dynamics of leukocyte presence during discrete phases of pancreatitis, and the role(s) of leukocyte-regulated pathways involved in pancreatic damage and repair. These studies will guide the identification of molecular targets for therapeutic intervention, with the goal of preventing PDAC development.
Christopher Loo, Ph.D.
Lund Lab
Cell, Developmental and Cancer Biology Department

Vaccinia Virus-Elicited Inflammation Induces Phenotypic Marker Changes in the Lymphatic Vessels of Mouse Ears

Christopher P. Loo, Jamie L. Booth, Tahsin Kahn, Jeffery Nolz, Amanda W. Lund

Abstract: Lymphoangiogenesis in melanoma is correlated with immunosuppression and poor patient outcome, yet the causative mechanisms are not clearly defined. Currently, little information is available on the general dynamics of lymphatic vessels (LVs) during a normal tissue environment, an inflammatory environment, and in resolution of inflammation. Using Vaccinia virus (VV) infection in the ear of mice, we investigated the cell surface phenotype of LVs during peak levels of VV titers and clearance of the infection. Interestingly, adhesion molecule ICAM-1 was upregulated on lymphatic endothelial cells (LECs) at day 7-30 post-infection (pi). T lymphocyte activation molecules PDL-1, CD86, and MHC-class II were also upregulated on LECs at day 7pi; however, expression returned to homeostatic levels by day 30 pi. The kinetics of CD8 T cells infiltrates into the infected ear were similar to PDL-1, CD86, and MHC-class II expression—a peak in frequency at day 10 pi and contraction by day 30 pi.
Thus, our data show LVs are responsive to the induction and resolution of inflammation by changes in immunomodulatory markers on LECs. This work supports that abnormal endothelial growth—a hallmark of tumor development—may support altered immune initiation, function, and retention.

Claudia Lopez, Ph.D.
Gray Lab
OHSU Center for Spatial Systems Biomedicine

State of the Art Electron Microscopy at OHSU

Chris P. Arthur¹ and Claudia S. López²

Abstract: The Multi-scale Microscopy Core (MMC) and FEI Living Lab at OHSU provides access to state-of-the-art electron microscopy (EM) imaging tools, including the Helios NanoLab™ 650 Dualbeam™, a combination scanning electron microscope (SEM) and focused ion beam (FIB) tool. With < 1.0 nm resolution in SEM, and <4.0 nm resolution in FIB, the Helios is capable of imaging large areas of fixed and embedded tissue samples in a semi-automated fashion, resulting in three-dimensional reconstructions. Our facility also houses two transmission electron microscopes (TEM), the Tecnai with iCorr™, and the Titan™ Krios™. The Tecnai is a 120kV TEM with an integrated fluorescence microscope. It is capable of fluorescent and electron imaging on the same sample without having to transfer between instruments. In addition, the Tecnai is a fully functioning TEM with cryo capabilities, able to acquire transmission images of negatively stained, plastic embedded and vitrified samples to a resolution of 5 Å. The Titan™ Krios™ is a 300kV TEM with cryo capabilities, STEM imaging, energy filter imaging, and direct electron imaging with a second generation Falcon direct detector (FEI). The Titan™ allows high resolution (<3.4 Å) imaging of single protein complexes, as well as tomographic imaging of vitrified cellular samples and complexes.

Shelley S. Mason and Caroline Enns

Abstract: Hereditary hemochromatosis (HH) is a common genetic disorder that causes increased iron absorption and accumulation in tissue and organs resulting in oxidative damage and organ impairment. In HH, gene mutations affecting iron regulation cause hyper-absorption of dietary iron resulting in ever-increasing body iron stores and paradoxically intestinal anemia. Iron-deficient enterocytes perpetuate intestinal iron absorption eventually exceeding the body’s iron storage capacity. The translation of ferritin is upregulated by iron through the iron regulatory protein (IRP)/iron response element (IRE) inhibitory system. In the absence of iron, IRPs bind IRE stem-loop motifs in the 5’ untranslated region of ferritin mRNA inhibiting translation. Because HH enterocytes have decreased ferritin, inducing the expression of intestinal ferritin to impede iron absorption may provide a potential therapy for HH and other iron overload syndromes. We propose several approaches to develop orally active IRE stem-loop IRP antagonists to induce intestinal ferritin expression, thereby blocking the uptake of iron into the body. Initial In vitro studies using a 3D culture system recapitulating intestinal crypt-villus architecture in an organoid-like structure (enteroids) demonstrated a physiological response to circulating Tf-bound iron. HH enteroids transfected with ferritin DNA and/or IRP antagonist are expected to impede iron absorption through a ferritin-imposed mucosal block.

Regulation of Wnt signaling regulates progenitor cell behavior during collective cell migration.

Hillary F McGraw and Alex V Nechiporuk

Abstract: To understand the modulation of Wnt signaling, we use the posterior lateral line primordium (pLLp), a cohort of ~100 cells that collectively migrate in the zebrafish embryo. The pLLp is comprised of proliferative progenitor cells and epithelial cells that form the posterior lateral line. Wnt signaling is active in the leading region of the pLLp and restricted from the trailing zone through expression of the secreted Wnt inhibitors dkk. We identified a mutant zebrafish strain, krm1nl10, which carries a mutation in the kremen1 gene, a co-receptor for Dkk proteins. Kreemen1 negatively regulates Wnt signaling by facilitating internalization of the Dkk-Kreemen1-Lrp5/6 complex. Disruption of Kreemen1 in the pLLp exhibited phenotypes associated with the loss of Wnt signaling, including downregulation of Wnt target genes, loss of cellular proliferation and survival. Transplantation of wild-type cells into the mutant primordia failed to rescue the krm1nl10 phenotype, revealing that the effects of Kreemen1 loss are cell non-autonomous; similar phenotype resulted from an ectopic activation of Dkk1b. Inhibition of dkk function partially rescues migration of the pLLP. We propose that Kreemen1 modulates Wnt activity by restricting a range of secreted Dkk proteins during collective cell migration of the pLLP, suggesting a previously undescribed mechanism for regulation of canonical Wnt signaling.
Abstract: As a central mediator of inflammation, the complement cascade is critical for opsonization, direct lysis of “damaged” cells, and induction of anaphylactoid reactions. Anaphylatoxins C3a, C4a and C5a induce migration of phagocytes, degranulation of mast cells and granulocytes, and relaxation of smooth muscle cells in “damaged” tissues. Because these responses are characteristic features of solid tumorigenesis, we speculate that complement also regulates chronic inflammation accompanying solid tumor development. To address this hypothesis, we used the K14-HPV16 transgenic mouse model of squamous carcinogenesis, where cancer development is dependent on humoral immunity and activation of FcRγ-signaling in infiltrating myeloid cells during premalignancy. Whereas C3-deficiency failed to alter carcinogenesis in HPV16 mice, absence of C5a receptors (C5aR) resulted in attenuated neoplastic progression. To determine the degree to which C5a is dependent on coagulation or fibrinolytic pathways that directly activate C5a, we examined the functional significance of urokinase (uPA) in HPV16/uPA⁻/⁻ mice, and revealed that in the absence of uPA (and plasmin), premalignant tissue failed to accumulate C5a and otherwise phenocopied HPV16/C5aR⁻/⁻ mice, with benign hyperplasias representing the most frequent terminal neoplastic phenotype. Together, these data indicate the significance of plasmin-activated C5a, and reveal that the complement pathway represents a tractable target for therapy.

Abstract: Altered blood flow during embryonic development has been shown to cause cardiac defects, however the mechanisms by which the resulting hemodynamic forces trigger heart malformation are unclear. This study used heart outflow tract banding to alter normal hemodynamics in a chick embryo model at HH18, and characterized the blood flow response with respect to the degree of band tightness. Optical coherence tomography was used to acquire 2D longitudinal structure and Doppler velocity images from control (n=16) and banded (n=25, 6-64% measured band tightness) embryos, from which structural and velocity data was extracted to estimate hemodynamic measures. Peak blood flow velocity and wall shear stress were increased in banded embryos (p<0.01) and dependent on band tightness. Remarkably, stroke volume in banded embryos remained comparable to control levels over the entire range of constriction (p>0.1). Characterization of the immediate hemodynamic adaptions determined that a controlled range of biomechanical forces can be produced in the early embryonic chick heart by varying the degree of outflow tract band constriction. This control is currently being used to investigate the effects of varied hemodynamics on tissue remodeling of the outflow tract wall and the subsequent cardiac defects that form later in development.
Asia Mitchell
Spellman Lab
OHSU Center for Spatial Systems Biomedicine

Non Random Chromosome Loss in Clear-Cell Renal Cell Carcinoma

Asia Mitchell¹, Suzi Fei¹, Nicholas Wang², Cathy D. Vocke³, W. Marston Linehan³, Paul Spellman¹

Abstract: TCGA reported 91% of clear cell renal cell carcinomas (ccRCC) exhibit loss of 3p, and 9% of this group exhibits whole chromosome 3 loss. Four of the five most frequently mutated ccRCC tumor suppressor genes are located on 3p, including VHL. Approximately 2% of ccRCC patients have inherited VHL mutations, resulting in VHL syndrome. CcRCC in VHL syndrome causes multiple bilateral tumors. We wish to understand if 3q loss is actively selected against. We whole genome shotgun sequenced multiple tumors from six VHL syndrome patients (42 total tumors) and performed copy number analysis. In four patients, we only observe 3p loss; while in one patient we observe whole chromosome 3 loss in 10 of 13 tumors, and in another, we identified whole chromosome 3 loss in two of six tumors. We observed distinct mutations in each tumor, supporting the hypothesis that these tumors are likely independent tumors. Additionally, we observed that breakpoints on chromosome 3 rarely occur beyond the PIK3CB locus. We propose that PIK3CB is haploinsufficient for tumor growth, and tumors with loss of whole chromosome 3 have germline variants, in cis, which can compensate for the haploinsufficiency.

John Muschler, Ph.D.
Gray Lab
OHSU Center for Spatial Systems Biomedicine

Endocytic trafficking of laminin is controlled by dystroglycan and disrupted in cancers.

Dmitri Leonoudakis¹, Ge Huang², Armin Akhavan¹, Jimmie E. Fata³, Manisha Singh¹, Joe W. Gray²,⁴, and John L. Muschler¹,².

Abstract: The dynamic interactions between cells and basement membranes (BMs) serve as essential regulators of tissue architecture and function in metazoans, and perturbation of these interactions contributes to the progression of a wide range of human diseases, including cancers. Here we reveal the pathway and mechanism for endocytic trafficking of a prominent BM component, laminin-111 (laminin), and their disruption in disease. Live cell imaging of epithelial cells revealed pronounced internalization of laminin into endocytic vesicles. Laminin internalization was receptor-mediated and dynamin-dependent, and proceeded to the lysosome via the late endosome. Manipulation of laminin receptor expression revealed that the dominant regulator of laminin internalization is dystroglycan (DG), a laminin receptor that is functionally perturbed in muscular dystrophies and many cancers. Correspondingly, laminin internalization was found to be deficient in aggressive cancer cells displaying non-functional DG, and restoration of DG function strongly enhanced the endocytosis of laminin in both breast cancer and glioblastoma cells. These results establish previously unrecognized mechanisms for the modulation of cell-BM communication in normal cells, and identify a profound disruption of endocytic laminin trafficking in aggressive cancer subtypes.
Damien Ramunno-Johnson
Vu Lab
OHSU Center for Spatial Systems Biomedicine

Using super-resolution quantum dot detection and automated cell segmentation to study subcellular processes

Damien L. Ramunno-Johnson, Wai Yan Lam, Joe W. Gray, and Tania Q. Vu

Abstract: The introduction of CCD cameras to microscopy changed the field and made it possible to introduce more quantitative imaging methods. One such method is localization microscopy, which gathers high precision position information for sub-diffraction-limited probes or markers. However, this location information becomes more useful if it can be put into context with other cellular structures, e.g. the plasma membrane, endosomes, and the nucleus. To relate the position of protein complexes to the plasma membrane, we developed image analysis software that efficiently determines the super-resolution location of proteins labeled with quantum dot probes (QD) in relation to the region of the cell plasma membrane, in 3D. The automated nature of this software significantly reduces the processing time from hundreds to two minutes per cell. This allows the collection of data from hundreds of cells, making it possible to perform detailed single protein-probe measurements in individual cells, as well extracting large-scale global behavior from an ensemble of cells.

Rachel Rigg
McCarty Lab
OHSU Center for Spatial Systems Biomedicine

Elucidating the role of circulating tumor cells in metastasis and thrombosis via biophysical characterization and transport modeling

Rachel A. Rigg, Kevin G. Phillips, Owen J. T. McCarty

Abstract: Metastasis is the leading cause of cancer-related deaths, and circulating tumor cells (CTCs) in the bloodstream are an indicator of metastasis as well as a potential cause of life-threatening, cancer-related thrombosis. A number of methods have emerged for isolation and characterization of CTCs, but questions remain about the role of biophysical parameters such as mass, volume, and density in CTC transport and survival in the vasculature. We employed quantitative phase microscopy (QPM) and Hilbert transform differential interference contrast microscopy (HTDIC) to determine cellular area, mass, density, and volume of CTCs isolated from patient samples via the high-definition CTC (HD-CTC) assay. This physical characterization of CTCs provides a specific phenotype that could yield new insights into the features of CTCs in different cancer types. Furthermore, we employed a mathematical model of CTC transport combined with temporal thrombin generation to elucidate the role of CTCs in thrombosis, showing an increased risk of coagulation where CTCs interact with the vessel wall or with other CTCs. In future work, this model will be modified to incorporate physical CTC parameters obtained from QPM and HTDIC to better predict CTC-vasculature interactions and identify patients most at risk for cancer-related thrombosis.
Abstract: Genetic, epigenetic and allelic differences provide unique phenotypes to tumor subpopulations, creating a landscape of differential drug sensitivity throughout the tumor. One such heterogeneous phenotype we observe within breast cancer is the heterogeneous expression of luminal, basal, and mesenchymal lineage markers by tumor cells. This differentiation state heterogeneity is pronounced in the basal-like subtype and is well modeled by basal-like breast cancer cell lines (BCCLs), where subpopulations of cells with luminal, basal, and mesenchymal features persist at specific population distributions.

To understand the therapeutic implications of differentiation state heterogeneity, we have run numerous basal-like BCCLs through high-throughput drug screens, and have quantified the changes in lineage marker expression, phenotypic distributions, and changes in cell number. Our results reveal that therapeutics, in a target dependent manner, induce robust alterations in both lineage marker expression and differentiation phenotype distribution within the cell lines. We focus on two classes of inhibitors, MEK inhibitors and dual-specificity PI3K/mTOR inhibitors, which both have robust, yet distinct effects on enriching cell phenotypes with reduced heterogeneity.

Our ongoing efforts are focused on identifying strategies that effectively eliminate the residual phenotypes we can therapeutically enrich, as well as understanding the mechanisms by which these therapies alter the differentiation phenotypes.

Abstract: Agents targeting the colony-stimulating factor (CSF)-1/CSF-1 receptor pathway are currently being evaluated in clinical trials for multiple types of cancer, either alone or in combination with chemotherapy. However, the mechanism by which macrophages sustain tumor growth and/or repress response to therapy is currently unclear. Here we show that macrophages within mammary carcinomas are a significant source of IL-10, and treatment with a blocking antibody against IL-10 receptor (IL-10R) was equivalent to CSF-1 neutralization in enhancing response to paclitaxel. Improved tumor responses were CD8+ T cell-dependent, but IL10 did not directly suppress CD8+ T cells or alter macrophage polarization. Instead, IL-10R blockade increased IL-12 expression by intratumoral dendritic cells, which was necessary for the enhanced chemotherapeutic response. In patient samples, expression of IL12A and cytotoxic effector molecules were predictive of pathological complete response rates to paclitaxel. These data reveal a novel role for the interaction between tumor macrophages and dendritic cells in mediating response to therapy, and expand upon a molecular pathway for targetable intervention.
**Nick Smith, Ph.D.  
Wong Lab  
Cell, Developmental and Cancer Biology Department  
*ePoster Presenter*  
The cancer stem cell marker CD166/ALCAM regulates homeostatic signaling within the normal intestinal stem cell niche  
Nicholas R. Smith, Trevor G. Levin, Douglas R. Keene, Paige S. Davies, Sidharth K. Sengupta, James Abe and Melissa H. Wong**

**Introduction:** The cell adhesion molecule CD166/ALCAM marks colorectal cancer stem cells and is highly expressed by malignant tumors, yet its role in disease is unclear. CD166 is normally expressed by crypt-based cells within the intestinal stem cell niche, suggesting that CD166 plays an important role in tissue homeostasis. We hypothesized that CD166 modulates regulatory signaling pathways within the niche.

**Methods:** We examined CD166 function within the intestine using a knockout mouse. Cellular proliferation, differentiation and signaling competency were analyzed by: Histo/immunohistochemistry, confocal and correlative light and electron microscopy, qRT-PCR, western blotting, and in vitro enteroid culture studies.

**Results:** Consistent with signaling function, CD166 was expressed by both Lgr5+ stem and Paneth cells within the normal niche. CD166 ablation dramatically altered Paneth cell homeostasis, causing decreased Lgr5+ stem cells. Interestingly, CD166-/- crypts contained more cycling progenitor cells, suggesting that CD166 regulates the balance between stem cell renewal and proliferation. Cultured CD166-/- intestinal enteroids grew more rapidly than controls and were less dependent on exogenous growth factors. Western blotting of crypt lysates revealed changes in regulatory signaling pathways.

**Conclusion:** These data indicate that the cell adhesion molecule CD166 coordinates critical signaling pathways within the niche that regulate stem cell function.

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**Anke Vermehren-Schmaedick, Ph.D.  
Vu Lab  
OHSU Center for Spatial Systems Biomedicine**

**Heterogeneous intracellular trafficking dynamics of brain-derived neurotrophic factor complexes in the neuronal soma revealed by single quantum dot tracking**

Anke Vermehren-Schmaedick¹, Thomas Jacob¹, Wesley Krueger², Damien Rammuno-Johnson¹, Agnieszka Balkowiec³, Keith Lidke², Tania Q Vu¹

**Abstract:** In the nervous system, growth factor-activated Trk receptor trafficking conveys biochemical signaling that underlies fundamental neural functions. Focus has been placed on axonal trafficking but little is known about growth factor-activated Trk dynamics in the neuronal soma, particularly at the molecular scale. Quantum dots (QDs) are intensely fluorescent nanoparticles that have been used to study the dynamics of ligand-receptor complexes. The current study establishes that QD conjugated brain-derived neurotrophic factor (QD-BDNF) binds to TrkB receptors with high specificity, activates TrkB downstream signaling, and allows single QD tracking capability deep within the soma of live neurons. QD-BDNF complexes undergo internalization, recycling, and intracellular trafficking in the neuronal soma. These trafficking events exhibit little time-synchrony as well as diverse heterogeneity in underlying dynamics that include phases of sustained rapid motor transport without pause as well as immobility of surprisingly long-lasting duration (minutes). Moreover, the trajectories formed by dynamic individual BDNF complexes show no apparent end destination; BDNF complexes can be found meandering over long distances throughout the neuronal soma in a circuitous fashion. QD-ligand probes are poised to provide understanding of how the molecular mechanisms underlying intracellular ligand-receptor trafficking shape cell signaling under conditions of both healthy and dysfunctional neurological disease models.
Abstract: One of the major obstacles to treating breast cancer patients with targeted therapeutics is the frequent occurrence of drug resistance. Much of this resistance can be attributed to intratumoral heterogeneity. Spatial heterogeneity within the tumor microenvironment results in differential contact with extra cellular matrix proteins, stromal cells, and signaling molecules, altering the intracellular signaling network of cancer cells depending on their location within the tumor. A shift in the signaling network can result in cancer cells adopting new signaling programs and patterns of gene expression. These differentiation events can endow cancer cells with physiological and morphological attributes that can aid them in proliferation and resistance to target therapeutics.

To address this we have employed cell spot microarrays to interrogate the functional outcomes that result from HER2+ breast cancer cells being exposed to various combinations of proteins common to the tumor microenvironment. These functional responses were measured by immunofluorescence assays following treatment with the HER2 targeted therapeutic lapatinib. The combination of HGF and Desmolgein II were identified in the array as causing complete resistance to the anti-proliferative effects of lapatinib, as well as modulating the expression of basal/luminal subtype differentiation markers keratin 14 and keratin 19.

Abstract: ZIP14 is a newly identified iron transporter with multi-transmembrane domains. In an attempt to dissect the molecular mechanisms by which iron regulates ZIP14 levels, we found that ZIP14 is endocytosed, extracted from membranes, deglycosylated and degraded by proteasomes. This pathway does not depend on the retrograde trafficking to the endoplasmic reticulum and thus does not involve the well-defined endoplasmic reticulum-associated protein degradation (ERAD) pathway. Iron inhibited membrane extraction of internalized ZIP14, resulting in higher steady state levels of ZIP14. Asparagine-linked (N-linked) glycosylation of ZIP14, particularly the glycosylation at N102, was required for efficient membrane extraction of ZIP14, therefore is necessary for its iron sensitivity. These findings highlight the importance of proteasomes in the degradation of endocytosed plasma membrane proteins and provide deeper insight into the mechanism by which ZIP14 is regulated.