Role of PITX2 in the Development of Intestinal Villi
Brian Aguilera

Shing Hu (BBS Cornell)

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PITX2 is a homeobox domain transcription factor that has been previously shown to have a very important role in gut morphogenesis. Interestingly, we have described how its asymmetric expression in the left dorsal mesentery is responsible for the initiation of gut tilting, looping and rotation. However, PITX2 is not only expressed in the mesentery in the developing embryo. It is also highly expressed in the endoderm which will later give rise to the cells of the intestinal villi and its role in this process has never been studied. Using a lineage tracing system in which Cre recombinase is driven by PITX2 and labels all cells that express PITX2 and their descendants with a fluorescent marker, we showed that PITX2 is first expressed very early on in the development of the intestinal lining at embryonic day 11.5, however its expression is limited only to the midgut. And if we take a look at later developmental stages PITX2+ cells give rise to many different types of cells in the villi including enterocytes, muscle cells and myofibroblasts, which are all important in the absorption of nutrients by the intestine in food digestion. Interestingly, when we looked at a hypomorphic mutation of PITX2, where an enhancer of PITX2 was knocked out, we noticed a very striking phenotype in both the lacteals and muscles in the villi of mutant pups. The lacteals of mutants appear to be short and stubby while in wildtype they are long and skinny and the muscles which normally recruit around the lacteals in wildtype seem unorganized in the mutants. Additionally when we looked at the livers of mutant vs wildtype pups we saw that mutant pups had an overaccumulation of fat in the liver even just a few days after birth. Given these intriguing results, I am very interested in determining when PITX2 is expressed in the endoderm and what kinds of cells PITX2+ cells give rise to as well as why PITX2 is necessary for correct villi formation and in what cells is its expression necessary.

MAGIC: Mosaic Analysis by gRNA-Induced Crossing-over
Sarah Allen

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Analyses carried out in chimeric animals have contributed to many fundamental discoveries in developmental biology and cell biology. Common techniques to produce â€œmosaicâ€ animals by mitotic recombination in Drosophila melanogaster rely on site-specific recombination systems such as Flp-FRT and require complicated genetic modification of chromosomes. Although useful in Drosophila melanogaster, these techniques are not generally available in other organisms. Here, we report the development of Mosaic Analysis by gRNA-Induced Crossing over (MAGIC), a new technique for the generation of mosaic animals using CRISPR/Cas9 and strategically placed gRNAs. This system produces double-strand breaks at specific sites in a chromosome, which can result in mitotic recombination between homologous chromosomes through homology-directed repair. We show that MAGIC efficiently produces homozygous clones in both somatic tissues and the germline in otherwise heterozygous animals. We further developed a toolkit to conveniently generate marked clones for genes located on chromosome arm 2L and demonstrated its application for gene function analysis in the wing imaginal disc and larval sensory neurons. MAGIC requires no genetic modifications of the chromosome of interest and thus can be used in unmarked, wild-type animals such as ones from the Drosophila Genetic Reference Panel (DGRP) collection. MAGIC can thus likely be applied to organisms beyond Drosophila, opening doors to novel studies in the broader field.

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ITK regulates the inflammatory/anti-inflammatory dichotomy in the development of Allergic Asthma
Orchi Anannya

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Introduction: Allergic asthma is characterized by the imbalance of pro and anti - inflammatory CD4+ T helper (Th) cells and regulatory T (Treg) cells. Differentiation into specific lineages of Th and Treg cells is dependent upon strength of T cell receptor (TCR) activation which induces specific signal transduction pathways that determine T cell identity. The interleukin 2 inducible T cell kinase (ITK) fine tunes this critical lineage determining TCR signal strength thus controlling balance of T cells.

Methods: We have used novel murine models that report the production of IL17A, IL10 and Foxp3+ Treg populations, along with selective ITK inhibitors, to investigate the role of ITK in the development of pro-inflammatory and anti-inflammatory CD4+ T helper (Th) cells and regulatory T (Treg) cells, and in the development of allergic asthma.

Results: We find that treatment of mice induced to develop allergic airway inflammation by House Dust Mite (HDM) with small molecule inhibitor of ITK (CPI) impaired development of allergic lung inflammation by impairing expansion of Th17, Th2 and Tr1 lineages, while enhancing Th1 Interferon gamma production and normalizing Th17/Treg ratio in the lungs. This was associated with reduced neutrophilic and eosinophilic recruitment to the lungs. Using cells cultured in vitro, we identified that inhibition of ITK causes cells cultured under Th17 differentiation conditions to switch to Foxp3+ Treg like cells instead of Th17 cells. Analogously inhibition of ITK caused cells cultured in Tr1 differentiation conditions to switch to IFNg+ Th1 like cells instead of Tr1 cells. Further characterization shows the Foxp3 positive Treg like cells resemble to Tregs and the IFNg+ Th1 like cells resemble Th1 cells based on phenotypic expression of lineage specific markers.

Conclusion: We have demonstrated that ITK is critical to generation of pro-inflammatory Th17 cells both in vivo and in vitro, and that treatment with ITK inhibitor can facilitate restoring the balance of pro-inflammatory Th17 and the anti-inflammatory Treg cell lineages in a murine model of allergic asthma.

Do Transposable Elements Play a Role in Primary Immunodeficiencies?
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Common variable immunodeficiency (CVID) is a late-onset primary antibody deficiency caused by defective B cell development. Recurrent bacterial infections frequently manifest in affected adult horses of different breeds, ages, pedigree, sex, and geographical region. Unfortunately, despite intense and expensive therapeutic management, patients ultimately die from septicemia or complications from infections. The etiology of CVID remains unclear. However, our laboratory has identified downregulation of B cell lineage-specific transcription factors E2A, PAX5, and CD19 in the bone marrow of CVID-affected horses. This disruption can be partially explained by hypermethylation of respective gene regulatory regions, but upstream genetic disturbances remain elusive to date. Non-coding gene sequences from endogenous retrovirus (ERVs) have been shown to interfere with normal B cell development. Accordingly, an ERV has been identified in leukocytes from CVID-affected horses. We hypothesize that derepressed retroviral sequences behave as transposable elements (TE) that unchain genomic instability for impaired B cell development. We aim to characterize genome-wide epigenetic alterations associated with equine CVID at a single-cell level, and to explore the possible implication of TE in the etiology of the disease. The chromatin accessibility and potential epigenetic aberrations related to CVID are being assessed through single-cell ATAC-Seq on peripheral blood and bone marrow samples. Additionally, single-cell RNA sequencing is being performed to obtain gene expression and activity of TEs. Integrated data analysis will be performed using computational tools to identify aberrant epigenetic landscape that results in faulty B cell differentiation. We anticipate that information derived from this project will uncover the role of TE in CVID pathogeny, and potentially guide the development of treatment approaches.

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Bone lysis and foreign body reaction following polyvinyl synthetic cartilage implantation
Jacqueline Chevalier

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Hallux rigidus or great toe arthritis negatively impacts 1 in 40 people over the age of 50. Hemiarthroplasty using a polyvinyl synthetic cartilage implant reports 90% success however, failure rates of 20% have been reported and the mechanism remains unclear. The purpose of this study was to characterize the pathologic processes at 6 months following hemiarthroplasty using the polyvinyl implant in an ovine model. An osteochondral defect was made in the medial femoral condyle in 6 sheep. In 4 sheep (A-D), a polyvinyl implant was placed per manufacturer recommendations. In 2 sheep the defect was left empty. Implant sheep C&D were euthanized at 1 & 5 months due to unrelenting pain; others were euthanized at 6 months. Post-mortem, joints were grossly evaluated, osteochondral and synovial membrane histology, and computerized tomography (CT) were performed. Gross evaluation of empty defect and implant sheep A&B revealed mild cartilage fibrillation on the femoral condyle and tibial surface whereas sheep C&D had severe fibrillation. On histology, the stromal reaction surrounding the implant was 3-fold wider in sheep C&D compared to A&B, and consisted of severe lymphoplasmacytic inflammation. Similarly, synovium in empty defect and implant sheep A&B had minimal cellular infiltration (1/4) compared to 4/4 in sheep C&D. Quantitative evaluation of CT scans showed that all sheep had some lysis and sclerosis with sheep C&D having 1.8-fold more lysis than the empty defect sheep. The evidence of bone lysis and foreign body reaction indicates an immune response to the implant and urges caution when considering use of polyvinyl implants.

Investigating the role of the Mojiang G attachment glycoprotein in membrane fusion and viral entry into host cells.
Eun Jin Choi

Mojiang virus (MojV) is a recently identified rat-borne virus classified in henipavirus genus within the Paramyxoviridae family, a genus that typically comprises bat-borne viruses. The attachment glycoprotein (G) binds to a host cell receptor and triggers the fusion F glycoprotein to undergo a conformational cascade that results in viral entry into cells and cell-cell fusion, a pathognomonic feature of paramyxoviral infections. MojV-G appears to be distinct from other henipaviral G proteins, as MojV-G only shares 22-24% sequence identity with the other known henipavirus G proteins. We hypothesize that MojV-G is structurally unique, but follows a similar membrane fusion and viral entry mechanism as compared to other henipaviruses. Here we found that MojV-G is structurally more stable as a tetramer in host cells as compared to another henipavirus, Nipah virus. Furthermore, we found that the three cysteine residues in the stalk domain of MojV-G (C141, C143, and C188) are important for maintaining such strong tetrameric structure. Interestingly, we also found that C141 inhibits cell-cell fusion in host cells, a novel and unique characteristic in the henipaviral genus. This study contributes to our understanding of MojV-G-induced membrane fusion, and of the diverse aspects of the mechanism of membrane fusion and viral entry amongst henipaviruses.

Left, right, and center: characterizing the asymmetric extracellular matrix of the dorsal mesentery and its effect on gut looping

Cora Demler

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Proper vertebrate gut development is heavily dependent on the evolutionarily conserved process of midgut rotation. The gut tube is suspended by the dorsal mesentery (DM), which has a left and right side with distinct cellular and molecular properties. The symmetry-breaking event in gut development is gut tilting, characterized by an expansion of the right DM and condensation of the left DM, creating asymmetric forces that push the gut tube to the left. We have shown that the right DM is enriched with the glycosaminoglycan hyaluronan (HA), and that this HA is covalently modified by the enzyme TSG6. This modified HA is necessary for the expansion of the right DM, gut tilting, and the exclusion of vascular progenitor cells from the right side. The left DM also has some level of HA in its extracellular matrix, but it is not modified by TSG6, is not sufficient to cause expansion, and is pro-vascular. How can the same molecule, HA, have opposite effects on vascular patterning in the left and right DM? One possibility is the presence of another HA binding partner/modifier on the left, opposing TSG6. We show that the left side of the chick DM expresses versican, a proteoglycan that binds HA and is known to be pro-angiogenic in other contexts. Versican is usually associated with an expanded matrix, unless it is cleaved by ADAMTS enzymes to form a condensed matrix. Given that we find versican on the condensed left side, we hypothesize that ADAMTS-mediated cleavage is occurring, and we show that ADAMTS9 is expressed in the left chick DM. In the mouse embryo, versican is found bilaterally in the DM, and it is cleaved after E10.5. I propose a model where distinct roles of HA on the left and right sides of the DM may be determined by its modification on the right by TSG6 and interaction with whole and cleaved versican on the left to critically regulate midgut rotation and gut vascular patterning.

In addition, we describe a novel midline structure made up of basement membrane in the middle of the DM. This midline barrier prevents the movement of diffusible signals between the left and right sides, although it is a transient structure and disappears after left/right asymmetric pathways have been set in motion. We hypothesize that the midline barrier is synthesized by the gut endoderm during development. Future work will determine if the midline barrier is critical for the establishment of asymmetry in the DM, just as the Lefty1-positive midline barrier is essential for setting up the left and right axis of the early embryo.

MicroRNA-10b is a regulator of cellular viability and proliferation in fibrolamellar carcinoma.
Adam Francisco

Adam Francisco, Andrew Massa, Matt Kanke, and Praveen Sethupathy

Fibrolamellar carcinoma (FLC) is a rare form of liver cancer that occurs mostly in adolescents without preexisting liver conditions. A genomic identifier of FLC is a ~400kb deletion on chromosome 19 which results in a fusion of two genes, *DNAJB1* and *PRKACA* (referred to as "DP"). The DP chimera is a functional kinase, the over-expression of which is tumorigenic in mice. Targeting DP pharmacologically has proven challenging; therefore, it is of critical importance to define downstream mediators of tumor phenotypes in order to identify candidate druggable targets. Genome-scale analysis of FLC tumors shows reproducible changes in gene expression. DP is likely to mediate gene expression through both transcriptional and post-transcriptional mechanisms, the latter of which includes microRNA mediated gene silencing.

In order to define aberrant microRNAs in FLC tumors, we performed small RNA-sequencing (small RNA-seq) in >25 tumors and 3 non-malignant liver samples. We identified multiple miRNAs significantly upregulated in FLC, including miR-182, miR-10b, miR-21, miR-449a, and miR-92b. We then compared the expression levels of these miRNAs to the levels reported in other cancers and identified the upregulation of miR-10b in FLC to be greater than any other tumor type. We also found that only miR-10b expression is further elevated in metastatic samples relative to primary tumor tissue. Finally, in a cell culture model of FLC, we found that only miR-10b expression is induced (greater than 10-fold) by DP activity. We hypothesized that miR-10b may function as a pro-survival, oncogenic factor in FLC.

To test this hypothesis, we performed loss-of-function experiments using miR-10b locked nucleic acid (LNA) inhibitors in a cell culture model generated from a patient-derived xenograft tumor. We found that suppression of miR-10b significantly lowered FLC cell viability and proliferation. We then performed an integrative analysis of chromatin run-on sequencing (ChRO-seq) and RNA-sequencing (RNA-seq) data to identify genes that are likely regulated by miR-10b in FLC tumor tissue. This analysis led to the discovery of *TRIM35*, or tripartite motif containing protein family member 35, which we validated in the FLC cell line. *TRIM35* is known to regulate the function of pyruvate kinase, which may explain the reduction of cellular viability when miR-10b is inhibited.

Our future directions include resolving the direct relationship between miR-10b and *TRIM35*, performing cellular migration and invasion assays after miR-10b inhibition, and developing and analyzing a miR-10b null FLC cell line. Additionally, we are identifying other oncogenic miRNAs with which miR-10b coordinates and directs FLC cell survival.

Targeting disease vector fertility: Utilizing the CRISPR/Cas13a system to silence potential fertility genes in male Aedes aegypti mosquitoes
Mark Gallardo

Mark Gallardo, Alexandra Amaro, Laura Harrington

Aedes aegyptiâ€¯mosquitoes are a major disease vector forâ€¯severalâ€¯medically important pathogens including yellow fever, dengue, and Zika viruses, which cause significantâ€¯globalâ€¯morbidity and mortality.â€¯With limited vaccines and antiviral therapies, vector control remains the focus of most Ae. aegypti-borne disease control efforts.â€¯Given the high frequency of insecticide resistance and the difficulty of controllingâ€¯Aedesâ€¯spp. with conventional strategies, understanding this vectorâ€™s reproductive and mating biology, with a focus on developing new control targets, is imperative.â€¯The CRISPR/Cas13aâ€¯systemâ€¯could be a useful functional tool to explore important reproduction genes.â€¯In theâ€¯ CRISPR/Cas13a system, a guide RNA (sgRNA) binds to the Cas13a enzyme and then guides it in targeting a gene ofâ€¯interestâ€™s transcript (mRNA)â€¯for cleavage. When compared to Cas9-mediated gene knockout, Cas13a isâ€¯a faster and less expensive methodology for functional gene screening. Given the intermittent functionality of RNAi in Ae. aegypti, Cas13a represents an attractive alternative gene knockdown modality. A gene of interestâ€¯that may reduce fertility inâ€¯Ae. aegyptiâ€¯males isâ€¯seminase (Ser 2), a trypsin-like serine protease found in the seminal fluid that is transferred to female mosquitoes during mating. In D. melanogaster this enzyme participates in an early regulatory stage of the post-mating process, initiating a protease cascade signaling pathway by hydrolyzing accessory gland proteins (LaFlamme et al., 2013). More recently, complete knockout of this gene in Bombyx mori and Plutella xylostella led to male sterility (Xu et al., 2019). Although this geneâ€™s role in male Ae. aegypti fertility remains unknown, we have recently identified potential homologs to this enzyme based on work in Ae. aegypti by Degner et al. (2019). Our ongoing experiments involve intrathoracic injection of the Cas 13a plasmid, demonstrating its expression, and targeting potential Ser 2 homologs by co-injecting designer CRISPR sgRNA. If effective transcript silencing is demonstrated, functional assays will be performed by mating gene-silenced males to wild type females and then monitoring female fecundity and fertility.

Mammary Cancer Extracellular Vesicles Drive Macrophage Coagulation and Reduce Antibacterial Immunity
Karla Garcia-Martinez

Karla GarcÃ-a-MartÃ-nez, Jingyi Chen, Chi-Yong Eom Ph.D. Tracy StokolÂ BVSc, Ph.D., DACVP, Nozomi Nishimura B.A., Ph.D., and Cynthia Leifer Ph.D.

Within the breast cancer tumor microenvironment (TME) there is battle between tumor cells, which promote their own growth and dampen antitumor immunity, and immune cells, which have the capacity to limit or eliminate tumor cells but are often suppressed. The most abundant immune cells found in the TME are macrophages, and multiple tumor cell-derived signals modulate macrophage function within the TME. Extracellular vesicles (EVs) are secreted by tumor cells and carry numerous proteins and molecules that control cell functions in the TME and at distant sites of potential metastasis. Previous studies showed that cancer EVs skew macrophages to an anti-inflammatory phenotype, and anti-inflammatory macrophages induce coagulation through the upregulation of procoagulant proteins. Since we previously showed that cancer EVs are themselves pro-coagulant, we used a model system of syngeneic mouse mammary breast cancer cells (E0771) with mouse bone marrow-derived macrophages in vitro to investigate whether mammary cancer EVs upregulate macrophage procoagulant activity and determine the effect of EV exposure on macrophage immune responses. We found that exposure to cancer EVs induces macrophage clotting and dampens intraphagosomal killing of bacteria. Mechanistically, this likely involves activation of the non-canonical inflammasome and disruption of phagosomal membranes. This study is significant because poor outcome for breast cancer patients is associated with hypercoagulation, increased incidence of thrombotic events, and increased susceptibility to surgical site bacterial infections after treatment surgery and prior to initiation of immunosuppressive chemotherapy. Our findings provide possible pathways to reduce cancer induced hemostasis and promote antimicrobial immunity.

A Veterinary Perspective on the most significant emerging viral pathogens.
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There is an interconnected relationship between animal health, human health, and the prosperity of the environment; the acknowledgement of this coexistence is termed One Health. As the status of the environment changes, emerging and re-emerging pathogens follow suit- leading to diseases that impact both humans and animals. (1) The World Health Organization (WHO), composed a list of the most severe emerging pathogens likely to cause â€œa public health emergency,â€ that lack concrete prevention and restorative care. (2, 3) The pathogens listed include: Crimean Congo hemorrhagic fever (Nairovirus), Ebola, Marburg, Lassa fever, MERS, SARS, Nipah, and Rift Valley fever viruses; all zoonotic, and all enveloped viruses. (2, 3) Given their pathogenicity, these and/or very similar viruses have a significant impact within the field of veterinary medicine. In an attempt to improve preparedness and response tactics for those whose lives and livelihood are closely linked with animals, we have categorized the significance of these viruses from a veterinary perspective. We looked at factors such as the species of animals infected, known treatment and prevention, mortality rates, geographic region and their economical significance to analyze the impact of these viruses within the field of veterinary medicine. These data were then used to quantify and rank the significance of these viruses globally. The ranking of viruses deemed most significant should aid the dedication of resources for research and development, to properly combat these pathogens on a global One Health scale.

Glucagon-like peptide-1 receptor signaling stimulates glucagon-like peptide-1 production and increases bihormonal insulin and glucagon positive cells in mouse and human islets
Marlena Holter

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Bariatric surgery, such as vertical sleeve gastrectomy (VSG), results in high rates of type 2 diabetes remission and leads to dramatic increases in meal-induced glucagon-like peptide-1 (GLP-1) secretion. GLP-1 regulates islet function by potentiating glucose stimulated insulin secretion (GSIS). We previously found that the beta-cell GLP-1 receptor (GLP-1R) contributes to improvements in glucose regulation and islet function in VSG-operated mice; and further, that VSG increases alpha-cell GLP-1 production by activating prohormone convertase 1/3 (PC1/3, gene: Pcsk1) expression in a beta-cell GLP-1R-dependent fashion. Thus, we hypothesize that beta-cell GLP-1R signaling enhances alpha-cell GLP-1 production to amplify GLP-1-induced GSIS in a paracrine positive feedback loop. To test this hypothesis, we first studied the impact of conditioned media generated from beta-cell GLP-1R WT and KO islets from sham or VSG-operated mice on alpha-cell gene expression. Conditioned media from islets isolated from VSG-operated mice increased alpha-cell Pcsk1 mRNA expression 3-fold in a beta-cell GLP-1R-dependent manner, suggesting that this effect is mediated by a secreted factor. We then determined whether this effect is specific to VSG or can be induced by pharmacologic stimulation of the GLP-1R. Twice daily Liraglutide treatment (200ug/kg) for 2 weeks in high fat diet-fed beta-cell GLP-1R WT and KO mice increased alpha-cell GLP-1 expression in WT, but not in KO mice. To assess the translational relevance of this data, we used an innovative single-cell RNA-sequencing platform, DART-seq, in human islets. Compared to saline-treated controls, liraglutide increased PCSK1, INS, MAFA, a beta-cell-enriched transcription factor, and decreased ARX, a driver

of alpha-cell maturity, in a subset of alpha-cells. Similarly, we found that conditioned media generated from liraglutide-treated islets increases alpha-cell Mafa mRNA expression compared with conditioned media generated from saline-treated islets. We further validated our DART-seq data by co-staining human islets for insulin and glucagon. Liraglutide increased alpha-cell GLP-1 expression and bihormonal insulin+ glucagon+ cells in human islets. To further validate our DART-seq data and determine the islet cell type through which liraglutide signals to induce bihormonal insulin+ glucagon+ cells we performed IHC on pancreas sections from liraglutide and saline treated beta-cell GLP-1R WT and KO mice. Liraglutide increased the percentage of bihormonal insulin+ glucagon+ cells in islets in a beta-cell GLP-1R-dependent manner. Together, these data demonstrate that the effect of beta-cell GLP-1R signaling to increase alpha-cell GLP-1 expression is mediated by a secreted factor, can be stimulated by pharmacological activation of the GLP-1R and has translational relevance in humans; and further, that the associated signaling pathway through which this occurs may prime alpha-cells for conversion to a more beta-cell-like phenotype.

Antimicrobial Resistant Bacteria Transmission between Humans and Companion Animals: A Scoping Review
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Objective: The transmission of antimicrobial resistant bacteria is a bidirectional pathway between humans and pets, and the household transmission of antimicrobial resistance (AMR) between humans and pets may be a significant public health problem. The objective of this scoping review is to identify the existing evidence of antimicrobial resistant bacteria transmission between people and pets (dogs and cats) globally.

Methods: A scoping review was used to determine the breadth of existing knowledge of the topic and map the body of literature. The searches were conducted through PubMed, Scopus, Web of Science, CABI Global Health and Networked Digital Library of Theses and Dissertations. The inclusion and exclusion criteria were made to assess and review the studies, all studies published in English and Mandarin that concentrated on AMR transmission between humans and pets (cats and dogs) are included in this review.

Results: This review captured 2,749 studies via PubMed, 2,394 via Scopus, 1,478 via Web of Science, 1,041 via CABI Global Health, and 163 theses, totaling 4,728 studies after de-duplication. The data extracted from the eligible AMR transmission studies is charted to identify information of the pet species, isolated bacteria species, the use of antibiotic, the means of transmission (direct contact or indirect contact), the sites of infection, the types of testing used in research, the locations of cases, and year of publication.

Conclusion: This scoping review identifies existing evidence and knowledge gaps of AMR bacteria transmission between pets and humans. It provides an overview of this topic for future scientific studies, policies, and public health interventions.

Th17 and regulatory T cells are inversely homed to an osteoarthritic joint // Regulatory T cells are unable to suppress the inflammatory effects of IL-1beta in an in vitro model of osteoarthritis

Laura Keller

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Disclosures: L. Keller (N), L. Fortier (N)

INTRODUCTION: The majority of patients with osteoarthritis (OA) have low-grade joint inflammation, which includes infiltration of CD4+ T cells into the synovial membrane and synovial fluid.1,2 In early OA, immunosuppressive T regulatory (Treg) cells are unable to suppress the pro-inflammatory effects of T helper 17 (Th17) cells and prevent the progression of disease. To test the hypothesis that in early OA, Th17 cells incite an inflammatory response but Treg immunosuppressive function is impaired, resulting in loss of joint homeostasis and to gain insight into the longitudinal progression of Th17 and Treg cells in OA, synovial fluid was collected from healthy equine joints that those at various stages of OA.

METHODS: Synovial fluid samples (n=155) were obtained from normal, early OA, or late/end stage OA joints. Synovial fluid and leukocytes were examined in 8 samples obtained from clinical patients, and 147 samples represented serial aspirates obtained from research horses. Samples were centrifuged at 1800 xg for 15 minutes. Supernatant was stored at -80ï‚°C for biochemical assays and in the 8 clinical samples, synovial fluid leukocytes were analyzed by flow cytometry for T helper cells (CD4), macrophages (CD14), and intracellular cytokine expression of IL-10 and IL-17A. The serial synovial fluid samples (n=147) collected from joints of an equine model of OA3 were analyzed using multiplex ELISA cytokine (IL-10, IL-17A, TNF-ï¡) and chemokine (CCL2, CCL5) assays. Cell population data from flow cytometric analysis were analyzed using one-way ANOVA with Tukeyâ€™s HSD post-hoc, and Luminex ELISA data were analyzed using a mixed model followed by Studentâ€™s T post-hoc test.

RESULTS: Flow cytometry of synovial fluid cells (n=8) revealed that in healthy joints, CD14+ macrophages were the most abundant leukocyte (47.4 ï‚± 10.6%), which significantly decrease in early OA (22.0 ï‚± 4.4%; $p<0.001$), and remain decreased into chronic disease (22.8 ï‚± 8.6%; $p<0.01$). Conversely, CD4+ T cells made up a small percentage of the leukocyte population (5.1 ï‚± 1.2%) within the healthy joint but were homed to the joint following injury (19.6 ï‚± 2.3%; $p<0.01$) and persisted into chronic disease (20.4 ï‚± 1.5%; $p<0.05$). There were few (5.8 ï‚± 1.7%) IL-10-producing lymphocytes in healthy synovial fluid, which significantly increased following injury (10.4 ï‚± 1.8%; $p<0.05$) but did not change into late OA (7.43 ï‚± 2.0%). There were also few (1.0 ï‚± 0.63%) IL-17A-producing lymphocytes in healthy and early OA synovial fluid (2.1 ï‚±2.6%) which significantly increased in late OA (7.4 ï‚± 6.1%). In the 147 serial samples, IL-17A concentration significantly increased at two weeks post-injury ($p<0.05$). CCL5 concentrations peak at 3 weeks post-injury and were significantly high than controls. CCL2 also peaked at 3 weeks post-injury and was significantly higher than controls at seven additional time points. IL-10 was significantly increased at 10 weeks post-injury compared to normal ($p<0.05$).

DISCUSSION: In early OA joints, there is an inverse relationship between macrophage and CD4+ T cell populations, which is likely a result of T cell homing initiated by CCL2 and CCL5 released from inflamed tissues of the joint.4 Additionally, the sustained release of CCL2 indicates that there is continued leukocyte recruitment to the joint that could contribute to the failure to return to homeostasis. Early enrichment of IL-10-producing lymphocytes in the joint following injury could be an attempt to diminish inflammation, but if not sustained it could indicate T cell exhaustion or T-cell phenotype switching and failure to resolve inflammation.5 This presence of IL-17A-producing lymphocytes in late OA is indicative of continued T cell homing and chronic inflammation, while the increase in IL-17A within the joint acutely following injury is likely a response to initial inflammation.6 Overall, these data suggest that Th17 and Tregs are both homed to the joint in early OA, but immunosuppressive Tregs are unable to suppress the pro-inflammatory effects of Th17 cells and prevent the progression of disease. Studies in progress aim to determine if Treg exhaustion or phenotypic plasticity are responsible for this failure of Tregs to resolve joint inflammation.

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Disclosures: L. Keller (N), L. Fortier (N)

INTRODUCTION: Arthritis is the leading cause of disability in the US.1â€“3 The role of T cells in the immunoregulation of osteoarthritis (OA) is relatively understudied. The continued progression of OA suggests that Treg suppressor functions are not sufficient to mitigate disease progression. This is in spite of early Treg migration to, and activation within the inflamed joint. To model the articular environment and study how Tregs affect joint homeostasis, a novel tri-culture system of Tregs, synoviocytes, and chondrocytes was developed. Our hypothesis was that, in early OA, Treg immunosuppressive function is not sufficient to mitigate joint inflammation following traumatic injury because of resultant exhaustion.

METHODS: To induce a Regulatory T cell phenotype, CD4+CD25high T lymphocytes were isolated from equine blood (n=1) using FACS and stimulated with Concanavalin A (ConA; 5 ï-g/mL), IL-2 (100 U/mL), and TGF-ï¢1 (2 ng/mL) for 4 days, and verified as Tregs by expression of Foxp3 and IL-10 using flow cytometry.4 Simultaneously, passage 1 synoviocytes were co-cultured in six-well plates with chondrocytes in transwells with a pore size of 0.4ï-m. To model normal and inflamed joints, co-cultures were +/- stimulated with of IL-1ï¢ (10 ng/mL) for 24 hours, washed with PBS, and media was replenished. Regulatory T cells were plated over the synoviocytes to create tri-cultures, and incubated for 24 hours. Synoviocytes and chondrocytes were analyzed for expression of IL-6 and MMP13, respectively using RT-qPCR.

RESULTS: Treg induction increased the population of CD4+CD25high from 11.2% of cells to 82.2%. There was not a minimal effect of Tregs on unstimulated synoviocyte IL-6 (1.2-fold increase) or chondrocyte MMP13 (1.1-fold increase). Stimulation with IL-1ï¢ increased expression of synoviocyte IL-6 (2.5-fold) and chondrocyte MMP13 (4-fold). Addition of Tregs to IL-1ï¢-stimulated co-culture increased expression of synoviocyte IL-6 (2.7-fold) and chondrocyte MMP13 (4.9-fold).

DISCUSSION: Tregs secreted IL-10 and expressed Foxp3+ indicative of an immunosuppressive phenotype, but were unable to mitigate the pro-inflammatory effects of IL-1ï¢ on chondrocytes and synoviocytes. In animal models, absence of IL-10 leads to more severe arthritis, but over-expression of IL-10 does not appear to mitigate disease progression in the long term. However, that data is focused on IL-10, and it is unknown if the full milieu of Treg cytokines would be sufficient to resolve inflammation.5,6 In this tri-culture model, even the embodiment of cytokines secreted by Tregs do not appear to be sufficient to restore joint homeostasis. This could be explained by the short duration of tri-culture, where Tregs do not have sufficient time to mitigate the effects of IL-1ï¢ on synoviocytes and chondrocytes. Alternately, the highly inflammatory environment of the tri-culture system could result in destabilization of Foxp3, leading to unsustained suppression functions.7 Further investigation into stability of Treg phenotype and function following tri-culture may reveal mechanisms responsible for failure of Tregs to resolve inflammation within the joint following injury. The results of these studies will inform on targeted immunomodulation for the treatment of OA.

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Neonatal-derived T regulatory cells impact the adult CD4+ T cell immune response to Mycobacterium tuberculosis
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Mycobacterium tuberculosis (Mtb), the causative agent of Tuberculosis (Tb), causes more mortality worldwide annually than any other single infectious disease. Despite the high prevalence of Tb, we lack an in-depth understanding of the immune response to Mtb, and have thus been unable to develop an effective vaccine. An unexplored component of the immune response to Tb is the ontogeny of the immune system. Neonatal humans succumb to a more fulminant form of Mtb as compared to adults, indicating that age-related differences in the immune system impact disease phenotype. To better understand immune ontogeny during Mtb, we used a CD4+ T cell fate mapper mouse model and fluorescently labeled CD4+ T cells produced during different stages of life. Interestingly, we found that neonatal CD4+ T cells persist into adulthood and are enriched for CD4+ FoxP3+ T regulatory (Treg) cells, which may suppress other immune cell populations. At baseline, neonatal-derived Tregs were more likely to downregulate CD62L (a marker of differentiation) and upregulate CD44 (a marker of activation), when compared to adult-derived Tregs. To determine a potential role for neonatal Tregs in the adult CD4+ Mtb response, we decided to analyze the immune response to Mtb in adult mice that had been given a CD4+ T cell depleting antibody in early life. In this way, we could compare animals lacking the neonatal layer of CD4+ T cells to those that had both neonatal and adult layers. We then infected both groups with Mtb. The CD4+ T cell response in the mice given the depletion antibody displayed more rapid kinetics. This work is significant because it suggests that the CD4+ T cell response to infection can be manipulated by targeting subsets of cells produced at different stages of development. Future work will examine the mechanisms by which neonatal-derived CD4+ T cells impact Mtb infection, such as how gene regulatory networks may cause increased activation of neonatal Tregs and subsequent suppression of other immune cell populations.

Dynamic Regulation of Chromatin Accessibility in Adipose Tissue in Response to Environmental Cues
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The excessive accumulation of fat leads to obesity, which is a major risk factor for many diseases, including type 2 diabetes. In contrast to white adipocytes which stores energy into fat, brown and beige adipocytes burn fat and generate heat. The activation of beige adipocytes, which can be induced in response to environmental cues within white adipose tissues, is considered to be a promising therapeutic strategy against obesity and metabolic diseases. Cold temperatures and Î²3-adrenergic receptor agonists have been used interchangeably to induce beiging but weather these two induce beiging differently remains unclear. We used single cell ATAC sequencing on adipose tissue from mice that exposed to cold temperature (6.5Â°C) or treated with Î²3-adrenergic receptor agonist CL-316,243 (1 mg/kg/mouse/day) for 1, 3, and 7days to elucidate the changes in cellular composition and chromatin states after cold and CL stimulation. The aims of this study are to understand the cellular and molecular changes during beiging and the underlying mechanisms of beiging induced by two different stimuli.

Survey of perceptions of international veterinarians regarding antibiotic use and resistance in dairy cattle
Sebastian Llanos Soto

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Continuous inappropriate application of antibiotics (ABs) represents a risk for the development of AB resistance in commensal and pathogenic organisms, particularly when they are applied at suboptimal concentrations. In this scenario, veterinarians have a crucial role in promoting measures to diminish the risk of AB resistance from emerging in dairy farms, as well as educating their clientele regarding the appropriate and responsible use of ABs. A questionnaire-based survey was designed and administrated to veterinarians in the context of the 2018 International Bovine Mastitis Conference held in Milan, Italy. Responses to the survey were analyzed using a combination of quantitative and qualitative analyses. Logistic regression was used to identify predictors of veterinariansâ€™ level of concern about the development of AB resistance on their clientsâ€™ farms and to compare perceptions of veterinarians working in the United States of America (USA) and European Union (EU). Responses about the reasons for overprescribing ABs by veterinarians were analyzed using thematic analysis. Participants perceived that nearly half of their clientsâ€™ overuse or inappropriately use ABs, and nearly half of their colleagues are overprescribing or inappropriately prescribing ABs. Participants concerned about AB resistant infections on the dairy farms they serve were more likely to have fewer years of experience in diary veterinary practice (Odds Ratio (OR)=0.91, 95% Confidence Interval (95% CI)=0.84-0.99) and consider better drug labelling to be important for reducing farmersâ€™ AB use (OR=6.86, 95% CI=1.21-38.93). No differences with respect to perceptions about AB use in dairy farming were observed between veterinarians working in the USA and EU. This study fills a gap in the understanding of perceptions of an international sample of dairy

veterinarians regarding AB use and resistance, particularly in those working in the USA and EU and proposes that age-focused initiatives might help improve knowledge about AB resistance emergence. Findings reported here will contribute to future research and aid in the development of strategies to improve AB use in dairy farming.

Multi-species comparison of miRNAs that regulate mammary stem and progenitor cell characteristics
James Miller

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The mammary gland is a defining feature of all mammals that, despite being highly conserved in structure and function, is remarkably variable in lactation strategies and disease incidence. Development and maintenance of the mammary gland is dependent on mammary stem and progenitor cells. These cells, while contributing to mammary gland function, including lactation, also are suspected to be a source of malignant transformation and carcinogenesis. When comparing mammosphere-derived epithelial cells (MDECs) from six mammalian species, we noted that these cells display differences in (i) mammosphere forming cells (MFC), a proxy for stem cell activity, (ii) colony-forming cells (CFC), a proxy for progenitor cell activity, and (iii) morphology. Micro-RNAs (miRNAs) are small non-coding effectors that post-transcriptionally regulate gene expression. To evaluate whether miRNAs could regulate some of these features in MDECs, we performed small RNA-Seq analysis and found significant expression differences for various miRNAs. The most notable miRNAs were (i) hsa-miR-92b-3p, which was highly expressed in species with MDECs that had low MFC and CFC, suggesting an anti-proliferative role, and (ii) hsa-miR-196b-5p, which was highly expressed in species with MDECs that had high MFC and CFC, suggesting a pro-proliferative role. Studying the function of these miRNAs in more detail will provide new insights into the regulation of important stem and progenitor characteristics, and may elucidate underlying molecular mechanisms regulating mammary gland functions in health and disease.

SIRT5 inhibition causes increased oxidative stress and impairs tumor progression and metastasis
James Mullmann

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The metabolic reprogramming that occurs in cancer cells creates dependencies that can be exploited for treatment. Our work establishes a new strategy to treat cancer by targeting a critical metabolic regulator, mitochondrial sirtuin 5 (SIRT5). SIRT5 is over-expressed in breast cancers and other malignancies, leading us to hypothesize that SIRT5 promotes cancer progression. We found that SIRT5 loss inhibits anchorage independent growth of human cancer cells. To examine how SIRT5 loss impacts tumorigenesis in vivo, we utilized MMTV-PyMT transgenic mice, which develop mammary adenocarcinomas and lung metastases. Sirt5 knockout (KO) MMTV-PyMT mice had increased survival, decreased tumor size, and delayed lung metastasis, as compared to Sirt5 wild-type (WT) MMTV-PyMT controls. Current studies are focused on the molecular mechanism by which SIRT5 promotes tumorigenesis and metastasis. SIRT5 KO cancer cells have reduced levels of important antioxidants such as NADPH and GSH, and higher levels of reactive oxygen species (ROS). These results indicate that SIRT5 KO cells experience greater levels of oxidative stress and suggest that SIRT5

could be promoting breast cancer by mitigating ROS. Importantly, pharmacological SIRT5 inhibition also impaired mammary tumor growth in both transgenic and human breast cancer xenograft mouse models. Considering that SIRT5 KO mice are generally normal, these data establish SIRT5 as a promising target for treating breast cancer.

Chemoproteomic Profiling of Gut Microbiota-Associated Bile Salt Hydrolase Activity
Bibudha Parasar

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The metagenome of the gut microbiome encodes tremendous potential for biosynthesizing and transforming small-molecule metabolites through the activities of enzymes expressed by intestinal bacteria. Accordingly, elucidating this metabolic network is critical for understanding how the gut microbiota contributes to health and disease. Bile acids, which are first biosynthesized in the liver, are modified in the gut by enzymes expressed by commensal bacteria into secondary bile acids, which regulate myriad host processes, including lipid metabolism, glucose metabolism, and immune homeostasis. The gateway reaction of secondary bile acid biosynthesis is mediated by bile salt hydrolases (BSHs), bacterial cysteine hydrolases whose action precedes other bile acid modifications within the gut. To assess how changes in bile acid metabolism mediated by certain intestinal microbiota impact gut physiology and pathobiology, methods are needed to directly examine the activities of BSHs because they are master regulators of intestinal bile acid metabolism. Here, we developed chemoproteomic tools to profile changes in gut microbiome associated BSH activity. We showed that these probes can label active BSHs in model microorganisms, including relevant gut anaerobes, and in mouse gut microbiomes. Using these tools, we identified altered BSH activities in a murine model of inflammatory bowel disease, in this case, colitis induced by dextran sodium sulfate, leading to changes in bile acid metabolism that could impact host metabolism and immunity. Importantly, our findings reveal that alterations in BSH enzymatic activities within the gut microbiome do not correlate with changes in gene abundance as determined by metagenomic sequencing, highlighting the utility of chemoproteomic approaches for interrogating the metabolic activities of the gut microbiota.

Phosphoproteomics Reveals a Distinct Mode of Mec1/ATR Signaling in Response to DNA End Hyper-Resection
Ethan Sanford

Vitor M. FaÃ§a, Stephanie C. Vega, William J. Comstock, and Marcus B. Smolka

The Mec1/ATR kinase is crucial for genome maintenance in response to a range of genotoxic insults, although how it promotes context-dependent signaling and DNA repair remains elusive. Here we uncovered a specialized mode of Mec1/ATR signaling triggered by hyper-nucleolytic processing (resection) of DNA ends. Cells lacking RAD9, a checkpoint activator and an inhibitor of resection, exhibit a selective increase in Mec1-dependent phosphorylation of proteins associated with single strand DNA transactions, including the ssDNA binding protein Rfa2, the translocase/ubiquitin ligase Uls1 and the HR-regulatory Sgs1-Top3-Rmi1 (STR) complex. Extensive Mec1-dependent phosphorylation of the STR complex, mostly on the Sgs1 helicase subunit, promotes an interaction between STR and the DNA repair scaffolding protein Dpb11. Fusion of Sgs1 to phosphopeptide-binding domains of Dpb11 strongly impairs HR-mediated repair, supporting a model whereby Mec1 signaling regulates STR upon hyper-resection to influence recombination outcomes.

TGFÎ² and BMP signaling synchronize the initiation of midgut asymmetry
Bhargav Sanketi

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The generation of asymmetry is fundamental to embryogenesis. We study the conserved counter-clockwise rotation of the gut as a model to understand left-right (LR) organ asymmetry. A critical aspect of this rotation is initiation of a leftward tilt directed by the conserved Pitx2 transcription factor, the master regulator of LR organ asymmetry. Failure to establish proper gut chirality leads to gut malrotation and catastrophic volvulus. The direction of gut rotation is specified by cellular and extracellular matrix (ECM) asymmetries within the dorsal mesentery (DM), which suspends the gut tube, and is downstream of Pitx2 expressed strictly on the DM left side. Although Pitx2 has been thoroughly studied in development, how asymmetric expression of Pitx2 in the gut is first established is unknown. Here, I uncover that TGFÎ² signaling is restricted to the left side of the DM and drives the timely activation of Pitx2 necessary for gut rotation. On the right side, BMP4 signaling initiates the expression of the enzyme Tsg6 and is the major rival of Pitx2. Tsg6 then triggers the accumulation of hyaluronan (HA) on the right, resulting in a dramatic expansion of the DM ECM, accelerating leftward tilting. Intriguingly, this right-sided expansion is sensed on the left side by the latent TGFÎ² complex, an extracellular feedback mechanosensor, resulting in ECM condensation ensuing deceleration. Surprisingly, Noggin, expressed by the gut tube primordium, antagonizes both the left and right programs, thus restricting morphological LR asymmetries to the DM. These data shed light on the major molecular players that not only break the initial symmetry within the DM but also synchronize the formation of the DM with its LR asymmetric deformation pivotal to gut rotation.

Suspending Cell Death-A Novel Stem Cell Response to Injury in Planarians
Divya Shiroor

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Stem cells are continuously exposed to multiple stresses, including radiation and tissue injury. As central drivers of tissue repair and regeneration, it is necessary to understand how their behavior is influenced by these stressors. Planarians have an abundant population of stem cells that are rapidly eliminated after radiation exposure via apoptosis. Low doses of radiation eliminate the majority of these stem cells, allowing a few to remain. Here, we combine radiation with injury to define how stem cells respond to tissue damage. We find that a variety of injuries induced within a defined window of time surrounding radiation cause stem cells to outlast those in uninjured animals. Injury stimulates localized cell death adjacent to wounds, in the same regions where stem cells persist. Surprisingly, stem cells persist in proportion to dying cells, without proliferating. Instead they are retained near the wound due to delayed apoptosis, which we quantify by combining fluorescence-activated cell sorting (FACS) with annexin V staining. Pharmacological inhibition of the extracellular signal-regulated kinase (ERK) prevents stem cell persistence after injury, implicating wound-induced ERK activity in this response. By combining radiation with injury, we have identified a novel stem cell response and uncovered a connection between dying cells and stem cells that remain. Future experiments will define mechanisms by which injury-induced cell death influences stem cell persistence. By delineating cellular interactions that govern stem cell function in planarians, we hope to uncover targets that could be used to enhance stem cell activity in more complex organisms.

Comparative segmental localization of GM1 in the epididymis: implications for wildlife conservation and contraceptive development
Danielle Sosnicki

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Although sperm maturation in the epididymis is essential for natural fertilization, very little is known about the molecular mechanisms that regulate sperm subcellular remodeling or acquisition of progressive motility. Further complications arise from differences in segmental epididymis histology across species. Improved understanding could both enhance assisted reproductive technologies as well as provide new targets for male contraceptives. Here, we investigated expression of bioactive lipids with particular focus on the ganglioside, GM1, across multiple species/taxa. This glycosphingolipid is known to play key roles in sperm capacitation and acrosome exocytosis, and is used as a biomarker for sperm fertilizing ability in human clinical medicine.

Direct fluorescence labeling was performed on tissue from mice, rats, domestic ferrets, domestic cats, dogs and rams. Confocal microscopy was used to visualize GM1 localization by labeling with the B subunit of cholera toxin. GM1 is highly expressed in segment 2 of the caput region of the mouse and in an apparently homologous segment of the domestic ferret caput epididymis. GM1 is present in distinct cell types in the epididymis of the rat but does not localize to a specific segment. The ram, dog and cat have expression that is not localized to a specific segment or to a distinct cell type, but instead has varying expression throughout the caput. Further investigation using mouse models has shown that GM1 is present in the form of GM1-enriched microvesicles that may be a population of epididymosomes involved in sperm maturation. Our findings provide a foundation for functional, mechanistic studies. Practical applications might include population control of target rodent species, reducing or eliminating the need to use current rodenticides which are problematic to non-target species, such as raptors. Additionally, species that have GM1 expression more similar to that of human may serve as a better animal model than the mouse.

Elucidating the mechanism of Dock7-mediated cellular transformation
Oriana Teran Pumar

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This research focuses on Dock7, a Guanine Nucleotide Exchange Factor (GEF) for the small GTPases Cdc42 and Rac1, and a novel player in the regulation of the PI3K/AKT/mTORC1 pathway. Studies in our laboratory showed Dock7 is essential for the transformed properties of several cancer cell lines, and that it interacts with Akt and other members of the pathway in co-immunoprecipitation assays. Furthermore, in comparison to the phosphorylation status of Akt in parental HeLa cells, Akt expressed in Dock7 KO cells does not undergo a stress-dependent phosphorylation Akt at either the S473 site nor the T308, indicative of a loss of its kinase activity. Accordingly, phosphorylation of downstream effectors of Akt is also downregulated. This research indeed highlights a versatile and important role for Dock7 in Akt regulation and a potential target for therapeutic intervention in tumors where Akt is hyper-active.

Utilizing genetically modified enteroids to identify miRNAs important for colon tumor development and progression
Jonathan Villanueva

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Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in the world. Somatic mutations drive colon tumor development by altering the activity of gene regulatory mechanisms that promote tumor growth and development. Different combinations of mutations can have unique effects on gene regulatory pathways, thereby promoting inter-tumor heterogeneity and affecting treatment outcomes. While studies have explored the impacts of individual oncogenic mutations on gene regulation, very few studies have systematically investigated how different combinations of mutations affect gene regulatory mechanisms. To address this gap, I propose to investigate how different combinations of driver mutations in CRC affect the transcription, expression and activity of microRNAs (miRNAs), as they have been well cited as important regulators of gene expression in CRC. I hypothesize that different combinations of oncogenic mutations lead to genotype-dependent and genotype-independent changes in the transcription of primary miRNAs (pri-miRNAs), through altered promoter and enhancer activity, resulting in altered activity of oncogenic and tumor suppressive miRNAs that control colon tumor growth and development. Understanding how mutation status affects miRNA regulators of colon tumor development will be important for the advancement of precision medicine by identifying candidate therapeutic targets for different mutational contexts of CRC.

Uncovering Escherichia coli multidrug resistance patterns among dogs with association rule mining
Ning Zhang

Craig Altier, Casey L. Cazer

Objective:

Multidrug resistance (MDR) among pets may put humans at a higher risk for MDR infections and also threatens the welfare of animals. The risk of non-foodborne sources of MDR transmission calls for more attention and unique approaches to reveal and predict resistance profiles in pets. This study uncovers MDR patterns in Escherichia coli isolated from dog urinary tract infections and shows whether the MDR patterns have changed over time.

Methods:

A veterinary diagnostic lab isolated 2,963 E. coli from dog urine between 2007 and 2017. We extracted one isolate per sample (n=2,493) that was tested by broth microdilution against a standard urine panel, which includes amoxicillin, ampicillin, ceftiofur, cefalexin, enrofloxacin, tetracycline, and trimethoprim-sulfamethoxazole. We analyzed MDR relationships in this dataset with a machine learning method called association rule mining, which extracts associations hidden among binary variables in large, sparse datasets.

Result:

Except for trimethoprim-sulfamethoxazole, the resistance prevalence trends for the other antimicrobials are similar over the ten years. Ampicillin has the highest overall resistance prevalence (32.2%), while trimethoprim-sulfamethoxazole has the lowest overall resistance prevalence (12.2%). The most prevalent resistance phenotype is pan-susceptible (N=1,570, 63%), followed by ampicillin-resistance only (N=128, 5%) and pan-resistant (N=101, 4.05%). Approximately 12.2% of samples (N=305) are resistant to three or more antimicrobial classes and therefore considered MDR. Association mining uncovers relationships between the antimicrobial resistances within each year and quantifies the strength of each association.

Conclusion:

Association rule mining is effective in uncovering potential MDR patterns and the change of the associations over time. The relationships between Î²-lactams, quinolones, tetracyclines, and sulfonamides will help clarify the MDR mechanisms in dog E. coli urinary tract infections and could indicate the potential for selection of MDR with common antimicrobial therapies.