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Micro RNA-10b promotes pathway of cellular survival and proliferation in Fibrolamellar carcinoma, a rare liver cancer

Adam Francisco

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Micro RNAs provide post-transcriptional regulation of gene expression by modulating messenger RNA abundance through the RNA-induced silencing complex (RISC). Through this mechanism, micro RNAs can act as oncogenes and tumor suppressors in context specific manners. We have performed small RNA sequencing to determine the expression pattern of micro RNAs in Fibrolamellar carcinoma (FLC), a rare form of liver cancer, and identified a cache of miRs consistently enriched. Of these candidate oncogenic miRs, we identified that miR-10b expression is further increased in metastatic FLC tumors and the expression of miR-10b is responsive to the activity of DNAJB1-PRKACA (DP), an aberrant kinase driving FLC formation.

MiR-10b is shown to promote cellular migration and invasion in aggressive cancers and we performed a pan-cancer analysis of miR-10b expression using data available through The Cancer Genome Atlas. We found that miR-10b expression is generally increased in hepatic and intestinal carcinomas and that miR-10b expression in FLC is greater than all other cancers types analyzed (but equal to expression in hepatocellular carcinoma).

We then performed miR-10b loss-of-function studies in a FLC patient derived xenograft (PDX) cell line using anti-sense oligonucleotide technology called locked-nucleic acids (LNA), which promotes miR-10b degradation and provides a non-genetic method of ‘knockdown’. Upon transfection with a miR10b LNA we observed a reduction in cellular viability and proliferation. Currently, we are in the process of identifying miR-10b targets by performing RNAseq analysis after knockdown and have future plans to perform in vitro migration and invasion studies.
**Next Top Model: Genomics and Transcriptomics Reveal Stark Differences in Nontyphoidal Serovars of *Salmonella enterica***

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In the United States, *Salmonella* causes approximately 1.2 million infections each year. The majority of these illnesses are the result of infection with nontyphoidal *Salmonella* (NTS), which cause gastroenteritis and diarrheal disease. There are over 2,600 *Salmonella* serovars, but *S. Typhimurium* is generally regarded as the model organism to study nontyphoidal disease. In this study, we analyzed the genomes and transcriptomes of three NTS serovars, Typhimurium, Javiana, and Cerro, to determine if Typhimurium is representative of other NTS serovars. We sequenced RNA extracted from *S. Typhimurium*, *Javiana*, and *Cerro* grown to late exponential phase in Luria-Bertani (LB) broth in triplicate. We also created pangenomes for each serovar using sequencing data from isolates on NCBI Pathogen Detection browser and determined gene presence or absence within and between serovars. Gene presence and absence analyses found 8,487 genes between all isolates included in this study, with 3,504 genes present in all isolates. All Cerro isolates have 3,878 genes in common, all Javiana isolates have 3,985 genes in common, and all Typhimurium isolates have 4,174 genes in common. Some of the genes present in only one serovar but not others, like cytolethal distending toxin (*cdtB*) in Javiana cause differing disease pathologies or adaptations to specific hosts. This study shows that *S. Typhimurium* is not necessarily the most representative serovar to study NTS. The three serovars studied here displayed major differences between their respective pangenomes, illustrating dissimilarities within the presence or absence of genes. The three serovars also exhibited differential gene expression, suggesting adaptations to their respective disease niches.
Detection of upper respiratory pathogens in cats at two animal shelters

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*Equal contributions

Animal shelters are a common source of pet cats in the United States. Maintaining individual cat and population health, despite population turnover is paramount. Upper respiratory infections in particular are a concern for shelter veterinarians and primary care veterinarians alike. Feline respiratory disease complex (FRDC) is considered an acute onset of clinical disease caused by feline herpesvirus-1 (FHV-1), feline calicivirus (FCV), Chlamyphilia felis, Mycoplasma sp., Bordetella bronchiseptica, and/or Streptococcus equi subsp. zooepidemicus. While feline coronavirus (FCoV) has been cited as a respiratory pathogen, the frequency at which this virus is found in the upper respiratory tract has not been quantified in either healthy or cats suffering from upper respiratory disease. In order to quantify which respiratory pathogens are present in healthy and sick cats, we have thus far enrolled 23 cats, of which 10 demonstrated upper respiratory disease and the remainder were considered healthy. Samples of the conjunctiva, oropharynx, and nasal cavity were tested for eleven pathogens: Panleukopenia, FCV, FHV-1, B. bronchiseptica, Chlamyphilia sp, influenza, Mycoplasma cynos, Mycoplasma felis, pneumovirus, S. equi subsp zooepidemicus and FCoV. Detected pathogens have included Panleukopenia, B. bronchiseptica, and M. felis. Enrollment is ongoing and will help understand the frequency of pathogens that contribute to respiratory disease in healthy and sick cats.
Understanding the remarkable chemosensitivity of testicular germ cell tumors

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Testicular germ cell tumors (TGCTs) are unusual among cancers in how they respond to DNA damage. Unlike other neoplasms, early stage TGCTs do not show spontaneous, oncogene-induced DNA damage response (DDR) activation, an important tumor suppressor mechanism in other tissues. Nevertheless, TGCTs mount a robust DDR to exogenous genotoxins, making them very sensitive to conventional chemotherapy, even after metastasis. To study the chemosensitivity and etiology of TGCTs, our lab developed the first genetically engineered mouse model of malignant TGCTs by inducing activation of Kras, an oncogene, and inactivation of Pten, a tumor suppressor gene, in germ cells using the CRE recombinase system. The tumors generated in these mice are malignant teratocarcinomas composed of pluripotent embryonal carcinoma (EC) and differentiated teratoma components and are very similar to the TGCTs commonly observed in men in terms of their embryonic onset, histopathological features, and chemoresponsiveness. Notably, the chemosensitivity of the TGCTs in our mouse model correlates with the exceptional sensitivity of their EC cells specifically, which are selectively depleted following chemotherapy treatment. To delineate the underlying molecular mechanisms, we established cultures of EC cells from our model and also differentiated them with various differentiation-inducing agents. Cultured EC cells were significantly more sensitive to cisplatin than their differentiated derivatives, mirroring the behavior of the corresponding cell populations in vivo. RNASeq analysis suggested that the mechanism underlying the chemosensitivity of EC cells and the chemoresistance of their differentiated counterparts was due in part to differential expression of genes involved in apoptosis and DDR pathways. Understanding the basis of TGCT chemosensitivity will inform the treatment of those tumors that do become chemoresistant and is also likely to aid in the development of treatments for the many other cancers that do not respond favorably to genotoxic chemotherapy.

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Synchronizing midgut formation with the initiation of its leftward tilt

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In early embryos, a single gut tube is modelled by various morphogenic events to give rise to the conserved looping of intestines. The direction of midgut rotation has long been assumed to be intrinsic to the tube itself. However, our research has challenged this paradigm and demonstrated that this rotation is instead driven by asymmetric cellular behavior within the dorsal mesentery (DM), an adjacent tissue that suspends the gut tube. The formation of the DM from splanchnic mesoderm is also accompanied by the remodeling of the underlying endoderm to create the gut tube. This process, in the chicken embryo, happens synchronously with the formation of the leftward tilt of the midgut, which triggers rotation. The chirality of this tilt is regulated by asymmetric cell organization and extracellular matrix (ECM) profiles across the left-right (L-R) axis of the DM, the disruption of which leads to gut malrotation and volvulus, a catastrophic strangulation of the gut vasculature. The glycosaminoglycan Hyaluronic Acid (HA) initiates this tilt by asymmetrically expanding the ECM on the right due to the timely modification of HA with heavy chain (HC) peptides, a process driven by the enzyme TNF-inducible gene 6 (TSG6). Whereas the LR transcription factor Pitx2 controls the left side of the DM, the molecular mechanism that trigger HA production and Tsg6 expression on the right remain unknown. I have identified unique expression domains of various Bone Morphogenetic Proteins (BMP), its regulators, and downstream effectors, which control ECM expansion unique to the right side of the DM synchronously with the remodeling of the endoderm and DM formation. Based on these data, I propose a model where the simultaneous antagonistic regulation of L-R signaling networks is the rate limiting event synchronizing midgut formation to asymmetric midgut rotation.

Key Words: Gut Looping, Lateral Plate Mesoderm, Hyaluronic Acid, BMP Signaling, Pitx2

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Immune cells are imperative for protecting humans against pathogens and abnormal cell growth. The ability of immune cells to proliferate and perform effector functions is limited by mitochondria, a major producer of energy, substrates, and metabolites. As we age and cells divide, mitochondrial genes accumulate mutations, which over time cause proteins to lose their functional capacity. Thus, protecting the mitochondrial genes from mutations is important for cellular function. The cells of the immune system undergo rapid cell division during an immune response, however, the effect of mitochondrial mutations on immune cells has been largely unexplored. My research is aimed at determining how mitochondrial gene mutations affect immune cells. Our general strategy is to perform experiments using a genetically manipulated mouse that carries a mutation in the "proofreading" exonuclease domain of PolG, PolG^{D257A}. This PolG mutant can still copy mitochondria DNA, but the polymerase cannot correct mutations. As a consequence, these mice harbor increased mitochondria mutations. My preliminary results show that increased mitochondrial DNA mutations can change immune cell functions. The results from this work is essential for advancing our understanding of the role of the mitochondria DNA integrity in immune cells.
Association Mining Identifies Patterns of Multidrug Resistance in Clinical Isolates of *Staphylococcus aureus*

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**Background:** Analyzing multidrug resistance (MDR) prevalence and trends is difficult with traditional statistical methods because MDR is a multivariate outcome and the number of possible resistance patterns increases exponentially with the number of antibiotics tested. Association mining, an unsupervised machine learning technique, can identify relationships between antibiotic resistance phenotypes in large, high-dimensional datasets by using the Apriori algorithm to efficiently search for resistance phenotypes that occur together.

**Methods:** We applied association mining to a dataset of 1,091 *Staphylococcus aureus* isolates prospectively collected from one New York hospital from 2008 to 2018 as part of the SENTRY surveillance program. Twenty antibiotics were included in our analysis: 9 beta-lactams, 3 fluoroquinolones, 2 macrolides, clindamycin, gentamicin, mupirocin, telavancin, tetracycline, and trimethoprim-sulfamethoxazole. Antibiotic susceptibility test results were classified as susceptible and non-susceptible based on CLSI breakpoints and EUCAST epidemiologic cut-off values. Combinations of resistance phenotypes, termed ‘itemsets’, were extracted from each year separately and filtered to maintain a false discovery rate ≤5% within each year. Itemsets were further filtered to eliminate any itemset that was a subset of another included itemset.

**Results:** Thirteen percent of isolates were susceptible to all antibiotics, 25% were resistant to 1 antibiotic class, 22% to 2 classes and 40% to ≥3 classes. Association mining identified 66 unique itemsets out of 1,048,576 possible combinations after filtering: 20 itemsets contained only beta-lactams, 3 contained only fluoroquinolones, 32 contained beta-lactams and fluoroquinolones, and 11 contained other antibiotic combinations. Resistance to clindamycin and tetracycline occurred together about twice as frequently as would be expected by chance, suggesting an association between these 2 resistance phenotypes. However, the prevalence of this resistance pattern was ≤5% in all years and only fulfilled the false discovery rate filter in one year.

**Conclusion:** Although some of the itemsets are congruent with known co-resistance and cross-resistance mechanisms in *S. aureus*, some are unexpected and merit further investigation. Association mining reveals relationships between resistance phenotypes in antibiogram datasets and could be used as a surveillance tool for monitoring established, or discovering emergent, MDR phenotypes.

**Acknowledgments:** This work is supported by the USDA National Institute of Food and Agriculture, Hatch accession #1013739. CC was supported by the Office of the Director of the National Institutions of Health, award #T32OD0011000. The content is solely the responsibility of the authors and does not represent the official views of the NIH or the USDA.
Building a low-cost Bluetooth-based sensor system for tracking of human movements

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Accurate tracking of employees’ approximate location is useful for a wide variety of applications, including healthcare. However, existing technologies have serious drawbacks in terms of high-cost and complexity. Our objective was to (1) develop a low-cost system for detection of room occupancy by employees and (2) evaluate its performance against manually logged truth data. To this end, we developed a prototype of a Bluetooth-based proximity sensor using a Raspberry Pi 3 B+ computer, programmed to detect employees’ mobile phones when nearby, allowing for passive positional observation without disrupting normal behavior. The detection software works by attempting to connect to devices using an assembled list of de-identified media access control (MAC) addresses supplied by volunteers. If the connection is successful, it requests the received signal strength indicator (RSSI) and stores it on a secure cloud server. To test the accuracy of the prototype we conducted 4 experiments in an environment consisting of 3 offices and a large teaching space with a sensor in each. During each experiment, 6-11 volunteers moved between the rooms at different schedules depending on the design of the particular experiment. Analysis involved assessment of the diagnostic accuracy in terms of correct detection of room occupancy. Overall, the prototype demonstrated high sensitivity (89%-99%) and specificity (85%-92%) in detection of individual room occupancy, with an overall accuracy of 87%-95%. These preliminary results support that the developed prototype has promising accuracy for tracking of employees’ location in addition to being cheap and easy to setup. Future plans include utilization of the prototype in healthcare to monitor and assess risk of nosocomial infections through employee movements.
Age-dependent changes of epigenome in somatic tissues in *Caenorhabditis elegans*

Cheng-Lin Li and Siu Sylvia Lee

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Epigenetic alterations occur as organisms age, and lead to chromatin deterioration, aberrant gene expression and genomic instability. Dysregulated epigenome has been linked to increased susceptibility to age-related disorders, including tumorigenesis. Therefore, this study aims to characterize the age-dependent changes of the epigenome and to understand epigenetic processes that drive aging phenotypes. The reversible nature of epigenetics makes it a favorable therapeutic target for promoting human health by maintaining a “youthful” chromatin state. Our lab has surveyed age-related changes in transcriptome and active histone marks (H3K4me3 and H3K36me3) in somatic tissues in *Caenorhabditis elegans* in previous publications (Pu, 2015 & 2018). In this study, we take a systematic approach by adding aging profiles of repressive histone marks (H3K9me3 and H3K27me3) and small RNAs. To this end, we have generated ChIP-seq data of repressive marks (H3K9me3 and H3K27me3) and small RNA-seq data in somatic tissues of young and old worms. Our results indicated a global reduction of H3K9me3 in peaks but also significant gain at specific regions during organismal aging. In contrast, H3K27me3 levels remain relatively stable. In general, boundaries of H3K9me3 and H3K27me3 peak regions are characterized by rises and falls in levels of repressive and active histone marks, respectively. These characteristic features of peak boundaries are blurred in H3K9me3 peaks but not in H3K27me3 peaks as worms age. We further noticed that H3K9me3 peaks that show significant gains correlate with binding profiles of H3K9me2-associated heterochromatic factors, and genomic regions marked with high levels of H3K36me3. Lastly, contrary to other organisms, we observed no global overexpression of repetitive elements (REs) in aged somatic tissues. Nevertheless, retrotransposable elements are over-represented among REs that are significantly upregulated with age. We are currently analyzing the age-dependent profiles of small RNAs (sRNAs) and how they might correlate with the expression changes of genes and REs.
Viral membranes sensitivity to general lipid peroxidators

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Only ten of the many medically relevant enveloped viruses are targeted by specific antivirals, which each tends to be specific for one or a few viruses. We and others are interested in broad spectrum antivirals against enveloped viruses, and several broad-spectrum antiviral compounds targeting virion envelope lipids have been developed since we developed the RAFIs in 2007. Some have been proposed to act by inducing lipoperoxidation under a model in which the metabolically inert virions would not repair lipid damage, thus resulting in their higher sensitivity to lipoperoxidation. However, the high protein content of envelopes may well inhibit the lipoperoxidation chain reaction.

We tested whether virions are particularly sensitive to lipoperoxidation, using well-characterized water- and lipid-soluble lipoperoxidators, AAPH and AMVN, respectively. The effects of the lipophilic AMVN on cell death and virion viability directly correlated with the extent of cell membrane or virion envelope lipoperoxidation, whereas the hydrophilic AAPH induced cell death and virion inactivation at lower concentrations than lipoperoxidation. Virion inactivation or envelope lipoperoxidation were only about 5-fold more sensitive to AMVN than cell death or cell membrane lipoperoxidation. Virion inactivation by incubation in aerobic conditions or exposure to presumed lipoperoxidant molecules was equally inhibited by vitamin E, cholesterol, or the BSA carrier, indicating that protection does not depend on antioxidant activity. The hydrophilic antioxidant vitamin C protected against aerobic conditions and known lipoperoxidators, but not against presumed ones.

In conclusion, HSV-1 virions are not particularly sensitive to lipoperoxidation, indicating that general lipoperoxidators do not show good antiviral potential.
Hyaluronan Plays a Two-Sided Role in Establishing Left-Right Asymmetry During Midgut Rotation

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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. While the left-sided transcription factor Pitx2 is often associated with the governance of organ laterality, we unexpectedly found that it is the accumulation of hyaluronan (HA) that breaks the symmetry of the DM, independent of Pitx2. Whereas HA is synthesized bilaterally in the DM, the enzyme TSG6 covalently modifies HA asymmetrically to make a stable complex of HA and heavy chain proteins (HC-HA) on the right side. HC-HA then triggers a dramatic expansion of the right DM, driving midgut rotation and creating an anti-angiogenic environment. In contrast, HA in the left DM is pro-angiogenic. Strikingly, degrading this left-sided HA decreases Pitx2 expression and makes the left impermissible to vascular development. Whereas Tsg6 is right-sided, the left DM produces versican, a pro-angiogenic sulfated proteoglycan that binds to HA—a relationship that is known to be essential in multiple developmental contexts but whose role in midgut rotation has not yet been characterized. I propose a model where distinct roles of HA on the left and right sides of the DM may be modulated by its modifications and binding partners to critically regulate midgut rotation and gut vascular patterning.
Elevated glycolytic metabolism limits the formation of memory CD8+ T cells in early life

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Neonates often develop poor immunity against intracellular pathogens. Since CD8+ T cells are essential for eliminating infectious agents, it is crucial to understand why they behave differently in early life. Previous studies have demonstrated that neonatal CD8+ T cells fail to form memory because of an intrinsic propensity to differentiate into short-lived effectors. However, the underlying mechanisms remain undefined. We now show that neonatal CD8+T cells exhibit higher glycolytic activity than adult CD8+ T cells after infection, which may be due to age-related differences in Lin28b expression. Importantly, when glycolysis is pharmacologically inhibited, the impaired formation of neonatal memory CD8+ T cells can be restored. Collectively, these data suggest that neonatal CD8+T cells are inherently biased toward undergoing glycolytic metabolism after infection, which compromises their ability to develop into memory CD8+ T cells in early life.
Injury promotes stell cell survival following radiation in planarians

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According to a prediction by the American Cancer Society, in the year 2018 cancer mortality is expected to cross 600,000 in the US alone. Increasing evidence indicates that radioresistant cancer stem cells that survive common therapies like radiation and cause malignancy contribute heavily to this burden. Based on recent results from our lab, we propose that studies in planarians could help uncover mechanisms of radiation resistance. Planarians are flatworms with phenomenal regenerative abilities, and entire animals can regenerate from tiny fragments within days. A large population of stem cells that responds rapidly to injury drives regeneration, but the specific cellular behaviors following wounding remain to be fully understood. One impediment is the sheer number of stem cells, that makes it difficult to discern changes in stem cell dynamics after injury. To manipulate stem cell numbers, we expose animals to ionizing radiation, which eliminates stem cells in a dose-dependent manner. We find that a strategically-timed injury prolongs stem cell survival in planarians subjected to radiation. Soon after radiation, stem cells accumulate near the wound site. Injured animals have a larger number of stem cells despite a complete absence of proliferation. Excitingly, wounding promotes stem cell survival even after exposure to an extremely high, lethal dose of radiation, when 100% of cells normally die. Our data therefore suggests that injury interferes with radiation-induced cell death, allowing stem cells to escape apoptosis and persist despite DNA damage. Current experiments are aimed at uncovering the mechanisms that promote injury-induced survival, which could also be relevant targets in the formation of radioresistant cancer stem cells in other animals.
How does public perception of antibiotic use on dairy farms contribute to preference for organic?

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Understanding the knowledge, attitudes and behaviors of the general public regarding organic farming and antibiotic use in animal agriculture is important, as these drive purchasing decisions, which in turn can ultimately affect antibiotic use practices in food animals. The aim of this study was to investigate the United States (U.S.) public’s perceptions of organic dairy farming practices and antibiotic use on dairy farms and to assess whether these perceptions may affect their purchasing decisions. Towards that end, we used data from the phone-based 2018 Cornell National Social Survey, developed in collaboration with Survey Research Institute at Cornell University. The survey collected information about participants’ (n = 1,000 U.S. adults): (i) knowledge of antibiotic use on dairy farms (conventional and organic) and (ii) preference for purchasing organic dairy products, as well as several explanatory variables and demographic characteristics. Data were analyzed using logistic regression. Preliminary results indicate poor knowledge of the general public about antibiotics, as 35.1% (351/1,000) of respondents considered the use of antibiotics in any kind of dairy farming to be illegal, while 20.0% (200/1,000) thought that antibiotic use for growth promotion is still permitted in the U.S. Participants who correctly identified the current regulations of antibiotic use on dairy farms to treat or prevent cow illness were more likely to oppose the use of any antibiotic ever in cows certified as organic (Odds Ratio = 1.52, 95% Confidence Interval = [1.11, 2.08]). Human health, including antibiotic resistance prevention, is the main reason to purchase organic dairy products (61.3%, 374/610). These findings underscore the existing misconceptions of the general public about farming practices and antibiotic use on dairy farms, as well as the importance of perceptions about organic products and antibiotics in their purchasing behavior.
IgE-binding monocytes in equine seasonal *Culicoides* hypersensitivity

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The most prevalent IgE-mediated allergic disease in horses is in response to *Culicoides* spp. salivary proteins. The disease mechanism is still not completely understood. In humans, a monocyte subpopulation expresses the IgE receptor Fc epsilon RI. These cells are associated with allergic diseases, but the role they play is unclear. Here we present the characterization of a similar population of IgE-binding monocytes in horses and the relationship of allergen exposure and clinical allergy with IgE-binding monocyte phenotype, prevalence and function. Peripheral blood mononuclear cells were purified from whole blood and analyzed by flow cytometry for monocyte surface protein expression and IgE binding monthly for one year. IgE-binding monocytes were enriched by magnetic sorting and were stimulated overnight with IgE crosslinking antibodies. Cytokine production was measured by a bead based Luminex assay. RNA was extracted from sorted cells for Fc epsilon RI subunit gene expression. Equine IgE-binding monocytes are IgE+ CD14+ MHCII++ CD163- and CD16-. The frequency of IgE-binding monocytes in the blood decreases during *Culicoides* exposure in both allergic and nonallergic horses. CD16 (Fc gamma RIII) expression is up-regulated during allergen exposure. IgE-binding monocytes express the trimeric form of Fc epsilon RI and do not express Fc epsilon RII (CD23), which is consistent with the corresponding population in humans. Purified IgE-binding monocytes also produce IL-10 upon IgE-receptor crosslinking, which may contribute to the functional role of these cells in the progression of allergic disease. In summary, IgE-binding monocytes have been identified and characterized in the horse and have differential CD16 expression depending on allergen. IgE-mediated IL-10 production suggests a potential regulatory role for these cells in the context of *Cul* hypersensitivity.
Chromatin dynamics and the transcriptional competence of HSV-1 genomes during lytic infections

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No antiherpesviral drug cures latency or prevents reactivation. New therapeutic approaches are thus needed. Epigenetics have been proposed to regulate HSV-1 replication, latency, and reactivation, and epigenetic inhibitors have been evaluated as potential antiherpesvirals. Ranylcypromine, pargyline, OG-L002, dimethylxalylglycine, ML324, 5′-deoxy-5′-methylthioadenosine, GSK126, GSK343, and UNC1999 inhibited HSV-1 replication or reactivation. However, they all inhibited the expected cellular epigenetic modifications, too, and were tested under models postulating the same epigenetic regulation for HSV-1 and cellular chromatin. It is thus difficult to envision epigenetic drugs as HSV-1 antivirals. HSV-1 and cellular chromatin may well be different, though. If so, any unique viral epigenetic regulation may be druggable. We used nuclease protection followed by chromatin fractionation and deep sequencing to probe for differences between HSV-1 and cellular chromatin. Like cellular DNA, HSV-1 DNA was protected to mono- to polynucleosome sizes in nucleoprotein complexes with the ratios of cellular mono- to polynucleosomes, in which it interacted with histone H3. However, HSV-1 and cellular chromatin were differentially accessible. Whereas most cellular DNA was in intermediately accessible chromatin, HSV-1 DNA was depleted in this chromatin and enriched in the most and least accessible chromatin. Therefore, viral and cellular chromatin have different dynamics. All HSV-1 genes were equally accessible under a given condition regardless of transcription levels. HSV-1 chromatin dynamics were similar to those of the cellular chromatin when there was not much viral transcription. We propose that the most dynamic HSV-1 chromatin is transcriptionally competent and the least, transcriptionally silenced. We are characterizing the mechanisms modulating the dynamics and transcriptional competence of HSV-1 chromatin to address which epigenetic regulators are involved in the regulation of HSV-1 transcription.
**Basophil of horses with *Culicoides* hypersensitivity produce IL-4 in response to allergens**

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*Culicoides* hypersensitivity (CH) is an IgE mediated allergic dermatitis in response to *Culicoides* allergens that typically develops at adult age. Clinical signs of allergy occur during *Culicoides* exposure in the summer and resolve in the winter. Interleukin-4 (IL-4) orchestrates the immune response of type 2 T helper cell during allergic reactions. We have previously shown that equine peripheral blood T-cells and basophils produce IL-4 in response to PMA and anti-IgE stimulation, respectively. Recent paradigm shifts suggest that basophils have a unique role in the regulation of allergic diseases. Here, we identified IL-4 secretion in PBMC after stimulation with allergen and analyzed the phenotype and numbers of IL-4 producing cells in CH affected and healthy horses. Eighteen horses (7 allergic and 11 non-allergic) were studied for one year. Heparinized blood samples were collected once a month for PBMC isolation. PBMC were stimulated with anti-IgE, *Culicoides* extract (Cul), or PHA. Basophil numbers in PBMC were evaluated by staining with basophil markers (IgE+MHCIIlow) and flow cytometric analysis. Phenotyping of IL-4 producing cells in CH affected and healthy horses was performed after stimulation of PBMC with anti-IgE, Cul and PMA/ionomycin in the presence of a secretion inhibitor. Cells were then analyzed for IL-4 production and with markers for basophils, monocytes, T-cells and B-cells. We demonstrated that allergic horses have higher basophil numbers and produce more IL-4 after Cul stimulation than healthy horses, while both groups secrete similar IL-4 amounts following IgE crosslinking. Moreover, Cul induced IL-4 was produced by basophils. In conclusion, peripheral blood basophils produce high amounts of IL-4 in allergic horses after stimulation with Cul allergens and allergic horses also maintain higher basophil numbers throughout the year.
Investigating the role of HCF-1 in lifespan and chromatin regulation in *C. elegans*

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HCF-1 (homolog of mammalian Host Cell Factor-1) is a highly conserved chromatin factor that acts as a potent regulator of longevity in the worm *C. elegans*. Mutation of *hcf-1* increases *C. elegans* mean lifespan by approximately 35%, however the connection between HCF-1’s action at chromatin and the lifespan extension observed in the mutant remains unclear. In mammals, HCF-1 acts to influence transcription and chromatin state by associating with transcription factors and several different chromatin modifying complexes, including the SIN3/HDAC complex that removes histone acetylation and the SET1/COMPASS complex that places H3K4me3. Immunoprecipitation mass spectrometry (IP-MS) analysis of *C. elegans* HCF-1 from our collaborators suggests that several chromatin modifiers may interact with HCF-1 in the worm, consistent with a key role for *C. elegans* HCF-1 at chromatin in the conserved SIN3 and possibly COMPASS complexes. Given these putative interactors and the connection of several of these chromatin modifiers to longevity regulation in *C. elegans*, we hypothesize that HCF-1 works at chromatin with conserved chromatin modifying complexes to regulate lifespan in *C. elegans*. To test this hypothesis, we are investigating whether the long lifespan of the *hcf-1* mutant is dependent on the presence of chromatin factors identified from our collaborator’s IP-MS analysis. Additionally, we have adapted a recently developed technique for obtaining DNA binding profiles with low cellular input, CUT&RUN, for use in *C. elegans*, where we will use it to profile HCF-1 binding sites and the consequence of *hcf-1* loss on the chromatin landscape.
Optimization model for spoilage reduction in the pasteurized fluid milk supply chain

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Capable of surviving high heat and pasteurization, spore-forming spoilage bacteria are the main culprit of milk waste due to microbial spoilage. They can speed up milk spoilage and make it unusable before its predetermined expiration date. However, the current pricing system for purchase of raw milk from producers (i.e., farms) does not involve testing milk for spore counts and does not consider any premiums for the quality of raw milk with regards to the spore counts. The goal of this study is to determine the optimal strategy for reducing milk loss due to spoilage. Specifically, we consider a novel flexible milk premium payment system based on milk’s spore counts in raw milk and two different existing spore reduction technologies (i.e., microfiltration and bactofugation) at the processing facility with the goal of achieving fewer spores in the final product (i.e., pasteurized raw milk packages) and therefore reduced or delayed milk spoilage at the retail. Our mixed-integer linear programming’s objective function is to maximize the milk shelf-life by selecting between different options (i.e., paying higher premiums, using one of the two spore reduction technologies at the processing facility, or a combination of these strategies) based on the limited available budget. Preliminary results show that the optimal solution is highly dependent on the size of the processor and their available budget, and the budget allocation in the optimal solution can extend the shelf-life up to five days. In conclusion, any processor can use our model and input their specific data and determine what the optimal solution for their supply chain is. This will allow them to optimally allocate their budget to increase their final product’s shelf-life.
Oxidative protein folding: A search for an alternative electron acceptor for endoplasmic reticulum oxidoreductin 1 (Ero1)

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About a third of all nascent proteins are secreted from cells. Many secreted proteins are stabilized by disulfide bonds, and disulfide bond formation is an essential part of the folding process. Disulfide bond formation is facilitated by a conserved pathway consisting of two proteins: PDI, which accepts electrons from protein thiols, and Ero1, which receives electrons from PDI and transfers them to a final electron acceptor. It has been established that oxygen can serve as the final electron acceptor for Ero1, and that this process generates hydrogen peroxide. Whether Ero1 can make use of alternative electron acceptors to minimize peroxide production as a byproduct of oxidative folding is unknown. Calculations suggest that excessive levels of cellular peroxide will be produced if every disulfide bond made generates one molecule of hydrogen peroxide. Moreover, the catalytic activity of the characterized enzymes that breakdown peroxide is anticipated to be insufficient to reduce these postulated peroxide levels. Altogether, these observations suggest that additional electron acceptors for Ero1 remain to be identified.

To identify proteins that interact with Ero1 and have a capacity to receive electrons, we affinity purified Ero1 and any associated proteins from yeast (S. cerevisiae). We identified several proteins involved in biosynthesis of ergosterol (the fungal equivalent of cholesterol). We have confirmed that Ero1 associates with several of the ergosterol (Erg) biosynthetic enzymes. Our working model is that the ergosterol biosynthesis pathway accepts electrons from Ero1, linking the processes of oxidative protein folding and sterol synthesis. We speculate that sending electrons from Ero1 to the sterol biosynthetic pathway will serve to limit peroxide production and any damage associated with elevated levels of this reactive oxygen species (ROS). We are currently testing how manipulation of oxidative folding may influence ergosterol biosynthesis, and also how disruption of ergosterol biosynthesis may impact disulfide bond formation.
NECAPs are negative regulators of the AP2 clathrin adaptor complex

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Eukaryotic cells internalize transmembrane receptors via clathrin-mediated endocytosis, but it remains unclear how the machinery underpinning this process is regulated. We recently discovered that membrane-associated muniscin proteins such as FCHO and SGIP initiate endocytosis by converting the AP2 clathrin adaptor complex to an open, active conformation that is then phosphorylated (Hollopeter et al., 2014). Here we report that loss of ncap-1, the sole C. elegans gene encoding an adaptin Ear-binding Coat-Associated Protein (NECAP), bypasses the requirement for FCHO-1. Biochemical analyses reveal AP2 accumulates in an open, phosphorylated state in ncap-1 mutant worms, suggesting NECAPs promote the closed, inactive conformation of AP2. Consistent with this model, NECAPs preferentially bind open and phosphorylated forms of AP2 in vitro and localize with constitutively open AP2 mutants in vivo. NECAPs do not associate with phosphorylation-defective AP2 mutants, implying that phosphorylation precedes NECAP recruitment. We propose NECAPs function late in endocytosis to inactivate AP2.
Monitoring and predicting the risk of nosocomial pathogen presence in a veterinary health-care setting

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There is a need for improved monitoring and surveillance of nosocomial pathogens in veterinary health-care facilities. We conducted an observational study of the environment and people (i.e., employees and students) in a large animal teaching hospital. Hospital rooms were evaluated for levels of shavings, hay & straw, feces, fur, other organic material, and moisture. Levels of moisture and organic material on cabinets, doorknobs, and computers were also assessed. Using an ethogram, we observed the presence of people in rooms, the presence of organic material and feces on clothing/shoes, interactions with animals, and handwashing behavior. Preliminary descriptive statistics for the floor indicated that the areas with the most animal traffic also had the highest amount of dirt and litter contamination. On average, wood shavings were present at the highest level, followed by hay & straw and moisture. An average 14.6% of people had organic material on their shoes, 12.1% had organic material on their clothing and only 8.2% were observed to wash their hands. There were an average of 14.8 animal “touches” per hour and one person was observed to touch 1.2 animals per hour. The most frequently touched animals were, in order, goats, pigs, sheep, horses, and cows. People spent, on average, 1.68 minutes at counters per hour. The wood shavings adhere to shoes and dispersed quickly and easily, which may explain why they were observed throughout the environment. We also conducted an agent-based modeling study of the hospital environment; a preliminary model was developed that assesses Salmonella spp. spread in the hospital following introduction of a hypothetical Salmonella infected patient. Overall, these preliminary results indicate that spread of nosocomial pathogens in a veterinary hospital might occur through floor littered with wood shavings and the poor hand hygiene.
SIRT5 Inhibition Causes Increased Oxidative Stress and Impairs Tumor Progression and Metastasis

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The metabolic reprogramming of cancer cells, termed the Warburg effect, involves aerobic glycolysis in which cancer cells ferment glucose and produce lactate. The metabolic alterations in cancer cells create dependencies that can be exploited therapeutically through targeting key metabolic enzymes. Our work establishes a new strategy to treat cancer by targeting a critical metabolic regulator, the mitochondrial Sirtuin 5 (SIRT5). SIRT5 regulates protein post-translational modifications on metabolic enzymes by catalyzing the removal of succinyl, malonyl, and glutaryl moieties. SIRT5 represents an attractive therapeutic target, as it is over-expressed in many cancers, including breast cancer. Therefore, we hypothesized that SIRT5 promotes breast cancer progression. We found that SIRT5 knockdown inhibits the anchorage independent growth of human cancer cells with little effect on normal cells. To examine how SIRT5 loss impacts tumorigenesis \textit{in vivo}, we utilized MMTV-PyMT transgenic mice, which are prone to mammary adenocarcinomas and lung metastases. SIRT5 knockout (KO) MMTV-PyMT mice had increased survival, decreased tumor size, and lacked lung metastases, as compared to SIRT5 wild-type (WT) MMTV-PyMT controls. Furthermore, pharmacological inhibition of SIRT5 impaired mammary tumor growth in both transgenic and human breast cancer xenograft mouse models. I am currently investigating the molecular mechanism by which SIRT5 promotes tumorigenesis and metastasis. SIRT5 KO cancer cells have higher levels of reactive oxygen species (ROS) and display lower levels of important antioxidants such as NADPH and GSH. These results suggest that SIRT5 KO cells are more susceptible to oxidative stress in cancer and hint that SIRT5 could be promoting breast cancer by mitigating ROS through one or more of its key metabolic substrates. My findings could open a new avenue for targeting cancer cells, and the proposed work will provide novel mechanistic insights into the role of SIRT5 in breast cancer progression.
SIRT1 loss promotes exosome production by triple negative breast cancer cells and mammary tumorigenesis in mice

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Despite increased awareness and improved 5-year patient survival rates for breast cancer, treatments for advanced breast cancer remain largely ineffective. Therefore, identifying novel therapeutic targets is of utmost importance. Several members of the sirtuin family of NAD+-dependent deacylase enzymes have been implicated in breast cancer. Sirtuin 1 (SIRT1) is known to act as a tumor suppressor, with its expression significantly downregulated in advanced, triple-negative breast cancers (TNBCs). Under normal conditions, SIRT1 stabilizes the mRNA transcript encoding ATP6V1A, a subunit of a V-type ATPase, through interaction with an RNA binding protein. Low SIRT1 levels causes degradation of ATP6V1A transcripts, and subsequent lysosomal dysfunction due to improper acidification. Consequently, breast cancer cells with low SIRT1 levels produce a greater number of exosomes, a subtype of non-classical secretory vesicles that are responsible for establishing the tumor microenvironment and pre-metastatic niche. We tested the hypothesis that low levels of SIRT1 promote tumorigenesis in vivo using MMTV-PyMT transgenic mice that are predisposed to spontaneous metastatic mammary adenocarcinoma. MMTV-PyMT mice treated with the SIRT1 inhibitor, Ex-527, had larger tumors and increased metastasis than the mice treated with the control vehicle, demonstrating that loss of SIRT1 function promotes tumorigenesis. These findings provide insights into the tumor suppressor functions of SIRT1 and suggest that SIRT1 activators may serve as effective therapeutics for breast cancer.
Bovine gammaherpesvirus 4 (BoHV-4)-based vectors for vaccine delivery in cattle

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Bovine gammaherpesvirus 4 (BoHV-4) is a member of the Gammaherpesvirinae subfamily. Given the ubiquitous nature of BoHV-4, this virus has been explored as vaccine delivery vector to various pathogens in diverse animal species. Here, our goal was to evaluate BoHV-4 as a vaccine delivery platform for cattle. We constructed two BoHV-4 recombinants, carrying the E2 glycoprotein of Pestivirus A 1b (BVDV-1b) or the hemagglutinin-neuraminidase (HN) protein of Bovine respirovirus 3 (BPI3) by inserting the heterologous proteins between BoHV-4 ORFs’ 1 and 2 via homologous recombination following infection/transfection of primary BT cells. Recombinant BoHV-4¹²/BVDV-E2 and BoHV-4¹²/BPI3-HN viruses expressing the BVDV-E2 or the BPI3-HN proteins were selected by plaque assays using the reporter green fluorescent protein (GFP). Selection and purification of the recombinants was assessed by PCR and IFA. The immunogenicity and protective efficiency of the recombinant viruses was evaluated in cattle. Twenty seven calves were allocated in three groups (T01 negative control, T02 BoHV-4¹²/BPI3-HN and T03 BoHV-4¹²/BVDV-E2; n = 9). The animals were vaccinated intramuscularly with 2 mL of the given recombinant virus (or DMEM, control) and evaluated daily. Animals from T03 developed high titers of neutralizing antibodies against BVDV-1b and BVDV-1a, while no NA were detected in animals from T01. At 35 days post-vaccination (dpv), the animals were challenged with BVDV. Animals from T01 presented elevated body temperatures and leukopenia, and higher frequency of viremia when compared to the vaccinated animals. At 56 dpv, the animals were challenged with BPI3. While no differences in body temperature were observed between the treatment groups, animals from T02 shed the virus for fewer days and at lower titers than animals from T01 group. Moreover, high NA titers against BPI3 were detected in animals following immunization with BoHV-4¹²/BPI3-HN. These results suggest that the BoHV-4-based vectors represent an excellent for vaccine delivery in cattle.
Novel paramyxoviral flow virometry and sorting techniques

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Paramyxoviruses highly relevant human pathogens that include the re-emerging measles (MeV), mumps (MuV), human parainfluenza viruses (hPIV) and emerging deadly henipaviruses. Despite vaccination regimens, MuV and MeV remain a health concern in developing countries. Additionally, there are no effective vaccine treatments for deadly emerging zoonotic henipaviruses. Paramyxoviruses thus remain a critical public health concern and a thorough understanding of virus structure and morphology will aid in the development of effective vaccines. Paramyxoviral virions range from several sizes depending on the virus (100 nm-1000 nm) and contain relatively large genomes for RNA viruses (15-19 kb). Embedded on the viral membrane are surface glycoproteins: the attachment protein (H, HN or G) and the fusion protein (F). Within the virion resides the matrix protein (M), nucleoprotein (NP), large protein (L) and the phosphoprotein (P). Structural factors that contribute to the infectivity potential of a virus remain to be elucidated for paramyxoviruses. This is because studies of individual viruses have only been recently possible with the advent of flow virometry, cryo-electron microscopy and electron tomography. For Junin virus, several structural components have been linked to greater infectivity potential. Specific genome segments of influenza have been reported to enrich surface glycoprotein content on virions. In paramyxoviruses, such as Nipah and Hendra viruses, the amount of glycoproteins on the cell surface affects fusion capacity. Therefore, virion structural components could play a role in infectivity in paramyxoviruses. Our lab has developed a flow virometry technique to measure surface glycoprotein content on NiV viral like particles as well detect conformational changes in the attachment protein. Using similar flow virometry tools, we will develop a novel viral flow sorting technique. This new tool will allow us to define a relationship between virion size and virion outer and inner morphology to viral infection using MeV as a model.
Inducing cAMP production in *Mycobacterium tuberculosis* is sufficient to cut off cholesterol catabolism

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Mtb’s continued existence depends substantially on its ability to acquire and utilize nutrients within the dynamic microenvironments it encounters in the human lung. A variety of studies have revealed that Mtb utilizes cholesterol and suggest that this carbon source is important to its long-term survival during infection. We discovered a collection of small molecules that inhibit Mtb’s growth in macrophages via a novel mechanism of action. These compounds disrupt cholesterol catabolism in Mtb at an early stage. Their effect is dependent on a mycobacterial adenylyl cyclase (Rv1625). The Rv1625 protein is rapidly activated by treatment with these compounds, resulting in a sustained super physiological increase in cAMP production by Mtb. Inducing cAMP independent of these compounds using a TetON system revealed that cAMP induction alone is sufficient to modulate cholesterol catabolism in a similar manner. We have also found that Rv1625 is required for certain steps of cholesterol catabolism, unexpectedly linking this protein directly to the cholesterol degradation pathway.
Differentially expressed synovial fluid metabolites and glycans in equine osteoarthritis

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Joint injury in racehorses frequently leads to post-traumatic osteoarthritis (PTOA). The aim of this study was to identify differentially expressed metabolic and glycosylation pathways in equine carpal PTOA. Long-term goals include enabling earlier diagnosis and identifying new potential therapeutic targets for joint disease. In one cohort, carpal synovial fluid (n=12; n=6 healthy, n=6 OA) was analyzed using high resolution liquid chromatography mass spectrometry. Data was analyzed using Principle Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), followed by pathway analysis. In a second cohort, (n=40; n=20 healthy, n=20 OA), carpal synovial fluid was analyzed using lectin microarrays and a lubricin sandwich ELISA.

Untargeted metabolomic analysis resulted in over 5,000 metabolites. Once adjusted for replicates and analyzed for significance (p<0.01), a total of 84 metabolites were found to be differentially expressed between healthy and OA joints. PCA and PLS-DA analysis exhibited separation between healthy and OA on the basis of differentially expressed metabolites. Of the remaining 84, 50 could be linked to Kyoto Encyclopedia of Genes and Genomes (KEGG) codes for pathway analysis. Histidine metabolism and vitamin B6 metabolism pathways were downregulated, and tryptophan metabolism was upregulated in PTOA. Neuraminate, muramic acid, and hexanoic acid were all significantly increased in OA.

Lectin microarray data identified distinct glycosylation patterns between healthy and OA synovial fluid samples, including increased core 1/core 3 O-glycosylation, increased α2-3 sialylation, and decreased α1-2 fucosylation in OA samples. Overall, O-glycans predominated over N-glycans in all synovial fluid samples. Synovial fluid lubricin was increased in OA joint samples approximately 10-fold as compared to controls (471.7 µg/mL +/- 153.2 µg/mL vs. 43.5 µg/mL +/- 7.0 µg/mL, respectively). These data suggest new potential diagnostic and therapeutic targets for equine OA; future targeted metabolomic and glycomic studies should be performed to verify these results.
Micro-RNA 29 regulates developmental differences in the human and mouse CD8+ T cell immune response

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Neonates are highly susceptible to infection and respond poorly to vaccination for reasons that are not well understood. Based on our published data, we believe neonates are particularly vulnerable to repeat infections because their naïve CD8+ T cells are intrinsically defective at differentiating into memory CD8+ T cells. To understand the underlying basis for these age-related differences, we performed small RNA sequencing and found that one miRNA in particular (miR-29) was selectively upregulated in adult CD8+ T cells (in mice and humans) and acting on their target genes in a predictable manner. We also performed adoptive transfer experiments and compared the ability of WT and miR29 KO donor CD8+ T cells to respond to infection. Interestingly, we found that donor CD8+ T cells lacking miR-29 secreted more effector molecules (IFNg, gzmB), expressed higher amounts of transcription factors (Tbet, Eomes) associated with effector cell differentiation, and failed to form certain subsets of memory CD8+ T cells (central memory, tissue resident memory), akin to neonatal CD8+ T cells. To translate these findings to humans, we employed a novel strategy to manipulate the expression of miR-29 in human CD8+ T cells. By packaging mimics and antagonirs into exosomes, we were able to age-adjust the expression of miR-29 target genes in naive neonatal (cord) and adult CD8+ T cells (PBMCs) and observed that antagonir EV treated human adult T cells preferentially proliferated and secreted effector molecules in response to TCR stimulus and oppositely for newborn T cells. Our research on miR-29 has the potential to uncover novel therapeutic strategies for enhancing the development of neonatal memory CD8+ T cells, and identify biomarkers for predicting how individuals respond to vaccination.
Recent work suggests mitochondrial dysfunction is an early response of chondrocytes to joint injury. In other tissues, mitochondrial dysfunction causes extracellular release of mitochondria-specific Damage Associated Molecular Patterns, including mitochondrial DNA (mtDNA), which perpetuate inflammation. mtDNA has not yet been explored in osteoarthritis. Our goals were to investigate if: 1) stressed chondrocytes release mtDNA in vitro, 2) articular injury increases synovial fluid (SF) mtDNA in vivo, and 3) mitoprotection affects SF mtDNA. Equine chondrocytes were cultured, then stressed with an inflammatory stimulus (IL-1β), or a mitochondria-specific inhibitor (oligomycin, FCCP, or rotenone/antimycin A). Media was harvested at 24 and 48 hours. Focal injuries were delivered to the articular surface of each talus of anesthetized horses (n=12). Both joints were treated with either SS-31, a mitoprotective peptide (n=6), or vehicle (control). SF was collected pre- and seven days post-injury. DNA was isolated from media and SF for mtDNA and nuclear DNA (nDNA) quantification. FCCP and rotenone/antimycin A groups released more mtDNA versus control and IL-1β groups at 24 hours, but returned to control levels by 48 hours, indicating mtDNA release is an early response. No differences in nDNA between treatment and control groups confirmed stimuli were non-lethal. Articular injury resulted in increased SF nDNA and mtDNA in untreated joints. SF nDNA and mtDNA after injury were significantly lower in horses treated with SS31 compared to controls. Results suggest chondrocyte MT dysfunction may trigger the extracellular release of mtDNA. In vivo, cartilage injury results in increased SF mtDNA, by selective release from live cells or via cell death/rupture. Treatment with SS-31 reduces SF mtDNA and nDNA to near baseline values, suggesting mitoprotection may prevent cell death after injury. These results are exciting because mtDNA represents a promising clinical biomarker, and mitoprotection represents a new therapeutic strategy for the treatment of early joint disease.
**Orf virus based vectored vaccine induces protective immunity to swine influenza virus**

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The parapoxvirus Orf virus (ORFV) encodes multiple proteins with known immunomodulatory property that are critical for virus virulence and pathogenesis. These immunomodulatory proteins are promising candidates for rationale design of safer and highly immunogenic viral vector platform. In this study, two ORFV recombinants targeting H1N1 subtype of swine influenza virus (SwIV) were generated by deletions of immunomodulatory genes of ORFV responsible for inhibiting activation of NF-kB pathway in the natural host. The first recombinant (OV-Δ121-HA1) was generated by inserting haemagglutinin (HA1) gene into ORFV121 locus, whereas the second recombinant (OV-Δ121-HA1-Δ127-NP) containing double gene deletions was generated by inserting haemagglutinin (HA1) gene in ORFV121 locus and nucleoprotein (NP) gene in ORFV127 locus. The immunogenicity of these recombinants was evaluated by intramuscular immunization of three groups of 3-week-old pigs; first group was sham immunized, second group was immunized with OV-Δ121-HA1, and the third group was immunized with OV-Δ121-HA1-Δ127-NP. Pigs were boosted 14 days post-vaccination and challenged with virulent SwIV strain A/swine/Ohio/24366/07 two weeks later. Pigs immunized with ORFV recombinants demonstrated high level of neutralizing antibodies against SwIV. Both the immunized groups showed high number of IFN-gamma producing T-cells, however IFN-gamma response was significantly higher in double recombinant (OV-Δ121-HA1-Δ127-NP) than the single recombinant (OV-Δ121-HA1). The pigs immunized with ORFV recombinants had significantly lower level of virus shedding in nasal secretions than the control group after challenge. Both ORFV recombinants protected pigs from SwIV induced lung lesions. Notably, protection conferred by double-gene deletion recombinant expressing both HA1 and NP proteins was higher than the single-gene deletion recombinant expressing HA1 alone. Overall, this study demonstrates the potential of ORFV virus as a vaccine delivery vector for swine influenza virus and development of other ORFV-vectored vaccines for swine.
Characterizing the Tissue Tropism of Equine Parvovirus-H (EqPV-H)

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Theiler’s disease, a.k.a serum hepatitis or idiopathic acute hepatic necrosis, is a devastating, highly fatal disease of horses. Recently, our research team identified a new parvovirus, Equine Parvovirus-Hepatitis (EqPV-H), as the likely cause of Theiler’s disease. The virus was present in samples from 27 of 28 consecutive Theiler’s disease cases (2014-2017) and hepatitis occurred in 8 of 10 horses experimentally inoculated with EqPV-H-positive serum, providing a strong link between this virus and Theiler’s disease. The study objective was to identify the cellular tropism of this novel equine parvovirus. Tissue samples were collected from 3 horses each during the acute and chronic stages of infection and analyzed for the presence of viral genetic material using qPCR. Tested tissues included serum, cerebrospinal fluid, liver, spleen, bone marrow, lymph node, lung, heart, salivary gland, small intestine, colon, kidney, synovium, and spinal cord. RNAscope in situ hybridization was used to confirm the presence of the viral genome within hepatocytes of formalin-fixed, paraffin-embedded liver tissue. Our qPCR data demonstrated a high viral load in the liver consistent with hepatotropism. In situ hybridization revealed large amounts of viral genomes within small numbers of scattered hepatocytes. A subset of infected hepatocytes displayed variable degrees of degeneration and necrosis. In one experimentally infected horse, hepatocytes in zones 2 and 3 of lobules were more affected. Our data indicate that EqPV-H is hepatocytotropic, providing further evidence for its etiological role in hepatitis and Theiler’s disease in horses.
A randomized trial to study the effect of automatic cluster remover settings on milking performance, teat condition, and udder health

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The objectives were to study the effect of 2 different automatic cluster remover settings on 1) milking characteristics, 2) milk component yields, 3) teat tissue condition, and 4) udder health. In a randomized controlled field trial, Holstein cows from 1 commercial dairy farm were allocated to 2 treatment groups. Treatment consisted of a cluster remover take-off milk flow threshold of 1.2 (ACR1.2) or 0.8 kg/min (ACR0.8) for 57 d. Milking characteristics (milk yield, MY; and milking unit-on time, MUOT) were obtained with electronic on-farm milk meters. Composite milk samples were collected and analyzed for fat, protein, lactose, and somatic cell count. Machine milking induced short- and long-term changes to the teat tissue condition were assessed visually. General linear mixed models demonstrated differences in MUOT, whereas no meaningful differences in MY were detected. Milk yield (least squares means, 95% CI) was 11.3 (10.9-11.8) and 11.3 (10.8-11.8) kg in groups ACR1.2 and ACR0.8. The effect of treatment on MUOT was modified by parity. Milking unit-on time in 1st, 2nd, and ≥ 3rd lactation cows, respectively, was 260.7 (252.0-269.4), 257.8 (247.4-268.1), and 260.2 (252.6-267.9) s in group ACR1.2; and 273.7 (264.9-282.5), 279.1 (269.4-288.8), and 295.7 (287.9-303.6) s in group ACR0.8. We detected no meaningful differences in milk component yields or linear somatic cell score. A generalized linear mixed model revealed an effect of treatment on machine milking induced short-term changes. The odds of short-term changes to the teat tissue were lower for cows in group ACR1.2 [odds ratio (95% CI) = 0.78 (0.63-0.96)]. No meaningful differences were detected in machine milking induced long-term changes between treatment groups. Increasing cluster remover take-off milk flow threshold from 0.8 to 1.2 kg/min decreased individual milking duration and alleviated machine milking induced short-term changes to the teat tissue without adversely affecting milking performance or somatic cell count.
Development of a murine model to study mitochondrial transfer from mesenchymal stem cells to stressed chondrocytes

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PURPOSE: Preliminary work from our group revealed the first evidence of MT transfer between MSCs and chondrocytes in vitro, using equine cells stained with fluorescent probes to track transfer events. Given the limitations associated with live-cell staining, our goal was to develop an in vivo model, utilizing transgenic mice expressing endogenous MT-targeted fluorescent proteins, to: 1) validate previous findings suggesting MSC-chondrocytes MT transfer in vitro, 2) quantify and characterize MT transfer events between cell over time and under various environmental conditions, and 3) allow future study of MT transfer in an in vivo model of PTOA.

METHODS: Chondrocytes from 5-day-old mCherry mice were cultured under normal (1g/L) or low (0.45g/L) glucose conditions, then stressed by the addition of IL-1β (1ng/ml), or a mitochondria-specific stressor 1) oligomycin (1μM), or 2) rotenone/antimycin (0.5μM/0.5μM). After 12 hours, chondrocytes were rinsed, and bMSCs from mitoDendra2 mice were added to chondrocyte cultures in 1:10 ratio. Cells were co-cultured for 12 hours, lifted, and fixed for flow cytometry. Experiments were duplicated on cover-glass slides, and live confocal 3-dimensional imaging was performed for 10 hours. Experiments were performed under both 5% and 21% O² conditions.

RESULTS: Time-lapse confocal imaging and flow cytometry data support MT transfer between bMSCs and chondrocytes in murine cells expressing endogenous fluorescent proteins. The percentage of green+ chondrocytes, red+ MSCs, and total double positive cells were significantly higher in normoxic than hypoxic conditions. Transgenic mouse lines enabled live-cell imaging to follow specific MT transfer events, as well as 3D localization of transferred MT within chondrocytes.

CONCLUSION: Consistent with data in other cell types and species, our findings suggest chondrocyte MT dysfunction initiates MT donation by MSCs in vitro. Intercellular MT transfer is a possible mechanism underlying the beneficial effects of therapeutically implanted MSCs.
Mitochondrial transfer from mesenchymal stem cells to intervertebral disc cells of sheep

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Previous research shows that placing mesenchymal stem cells (MSCs) into experimentally induced intervertebral disc (IVD) lesions prevents degeneration; however, the mechanisms underlying the beneficial effects of implanted MSCs remain unclear. One possible mechanism is mitochondrial (MT) transfer from MSCs to damaged IVD cells, which restores MT function to repair tissues. This phenomenon is known to occur in chondrocytes in vitro and alveolar epithelial cells in vivo. Recent evidence suggests that tunneling nanotubules mediate MT transfer from sheep MSCs to IVD nucleus pulposus cells in vitro. The goal of this study was to investigate MT transfer between sheep MSCs and IVD annulus fibrosus (AF) cells in vitro and ex vivo. Preliminary experiments aimed to transfect bone marrow-derived MSCs from sheep sternebra with a MT-specific GFP using electroporation. AF cells were transduced with a cytoplasmic RFP using a lentiviral vector. Both cell types were grown in cell culture flasks at 37°C/21% O₂/5% CO₂. Successful transduction of AF cells and transfection of MSCs was observed using confocal microscopy. These cell lines will be used in future experiments to track potential MT transfer in-vitro. AF cells will be stressed for 12 hours using a general inflammatory stimulus, interleukin-1β, to simulate disease. The AF cells will then be co-cultured with MSCs for 12 hours. Flow cytometry will quantify the percentage of AF cells that gain GFP fluorescence, indicating MT transfer, while confocal microscopy may demonstrate the phenomenon qualitatively. MT transfer from stem cells to injured AF cells resulting in improved tissue repair represents a promising future for treatments of IVD degeneration and other orthopedic diseases.

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An Induced Pluripotent Stem Cell Model of LMNA Dilated Cardiomyopathy to Study the Disease Pathogenesis

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The LMNA gene encodes the intermediate filament proteins Lamins A and C, which form the nuclear lamina: a meshwork inside of the inner nuclear membrane that gives the nucleus structural support and modulates the organization of chromatin and other nuclear proteins. LMNA mutations cause numerous diseases, termed laminopathies, including dilated cardiomyopathy (LMNA-DCM) and muscular dystrophies. LMNA-DCM involves dilation of the left ventricle and thinning of the ventricle walls, which weakens the heart. On the cellular level, LMNA-DCM and skeletal muscle laminopathies are characterized by structural defects resulting from mechanically weakened nuclei that cannot adequately respond to mechanical forces, and by disrupted signaling pathways; however, the precise molecular mechanisms underlying the disease pathology remain unknown. In vitro models of LMNA-DCM are lacking, because primary cardiomyocytes are challenging to isolate and short-lived in culture. Thus, we aimed to develop an induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) model with LMNA-DCM patient-derived cells to study the cellular pathology. We found that LMNA-DCM iPSC-CMs have increased nuclear deformities compared to healthy controls, including abnormal nuclear shape and nuclear envelope blebbing, recapitulating defects found in cardiac tissue of laminopathic models. Furthermore, using a nuclear rupture reporter system, in which a GFP is fused to a nuclear localization signal (NLS-GFP), we observed nuclear rupture in LMNA-DCM iPSC-CMs, indicated by NLS-GFP spilling into the cytoplasm. Nuclear rupture exposes nuclear material, including chromatin, to the cytoplasm and can cause DNA damage. Accordingly, we found that LMNA-DCM iPSC-CMs had increased levels of γH2AX, a marker of double stranded DNA breaks. In conclusion, our work demonstrates that patient-derived iPSC-CMs serve as a valuable in vitro model of LMNA-DCM. Ongoing work is aimed at investigating structural damage to nuclei, as well as altered signaling pathways and DNA damage, in hopes of finding potential treatment avenues for LMNA-DCM.
Equine herpesvirus 1 (EHV-1) is an easily transmitted respiratory pathogen that impacts horse health globally. Utilizing cell-associated viremia in peripheral blood mononuclear cells, EHV-1 can be transported from the respiratory epithelium to target tissues, such as the central nervous system (CNS). Viral replication within CNS endothelial cells results in nervous system disorders via vascular damage and is often fatal. Our previous studies have shown that cell-associated viremia is completely prevented in protected horses and that protection correlated with systemic and intranasal antibody amounts against EHV-1. In a protected horse, viral entry and/or viral replication in respiratory epithelial cells was also inhibited. We hypothesized that intranasal neutralizing antibodies are essential to prevent respiratory epithelial cell infection and all down-stream of EHV-1 including cell-associated viremia. In an effort to better characterize the mechanisms of protection against negative effects of EHV-1 replication, we investigated the viral neutralization ability of equine IgA and individual IgG antibody isotypes (IgG1, IgG4/7, IgG5, and IgG6) purified from nasal secretions. Fast Liquid Protein chromatography (FPLC), was utilized to isolate the antibody isotypes from nasal secretions. Purification was followed by isotype verification using an EHV glycoprotein specific multiplex assay. Lastly, a neutralization assay was performed using the various antibody isotypes to determine the lowest concentration at which the antibodies are able to neutralize viral cytopathic effects. From our work, we observed that IgG4/7 and IgG1 are most efficient at protecting infection of the cells in vitro, thus are likely involved in protecting horses in vivo. In conclusion, intranasal neutralization of EHV-1 by IgG1 and IgG4/7 antibodies provides a mechanism for protection against viral entry and/or replication. These findings have implications for vaccine development and prevention of future EHV-1 outbreaks.
Using RNA in situ hybridization as a more highly sensitive method for pathology-based diagnosis of feline infectious peritonitis as compared to immunohistochemistry

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Feline infectious peritonitis (FIP) is a fatal disease in cats caused by a mutated form of feline coronavirus. Two serotypes of FIPV exist: type 1 viruses constitute 85% to 95% of FIP cases, while type 2 viruses are responsible for the remaining 5% to 15% of infections. Immunohistochemistry (IHC) currently serves as the gold standard for diagnosis of FIPV within tissue. However, IHC has some limitations, such as relatively low specificity and a wide variation in sensitivity. In situ hybridization (ISH) targeting viral RNA has an established foothold in infectious disease diagnostics and presents a potentially improved method for detection of FIPV. This study sought to evaluate the efficacy of RNA ISH probes targeted to FIPV, as compared to IHC using monoclonal antibody FIP 3-70. Formalin-fixed paraffin-embedded tissues from FIP-positive cats were used for ISH, with RNA presence determined colorometrically. ISH tissue slides were then compared to their IHC counterparts, with efficacy determined based on metrics including staining intensity and abundance of stained cells. Positive ISH staining on tissue was found to be more intense and abundant than for IHC—suggesting that ISH serves as a more sensitive method for the detection of FIPV in comparison to IHC.
ITK regulates the Th17/Treg dichotomy in the development of Allergic Asthma

Orchi Anannya and Avery August

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Allergic asthma pathogenesis is characterized by the imbalance of pro-inflammatory and anti-inflammatory CD4+ T helper (Th) cells and regulatory T (Treg) cells. Differentiation of the naïve CD4+ T cell into specific lineages of Th and Treg cells is dependent upon initial strength of T cell receptor (TCR) activation which activates specific signal transduction pathways ultimately leading to expression of lineage specific transcription factors. The interleukin 2 inducible T cell kinase (ITK) fine tunes this critical lineage determining TCR signal strength thereby determining T cell identity. We have used novel murine transgenic models that report the production of IL17A, IL10 and Foxp3+ Treg populations, mice expressing a unique ITK allele sensitive variant (ITKas), along with ITK specific 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. We find that inhibiting ITK in T cells (using a small molecule inhibitor of WT ITK, CPI), or in T cells expressing ITKas (using ITKas inhibitor MBPP1), selectively inhibits differentiation of pro-inflammatory Th17 cells while inducing the generation of a population of cells resembling Foxp3+ Tregs. Furthermore, treatment of mice induced to develop allergic airway inflammation by exposure to House Dust Mite (HDM) allergen with small molecule inhibitor of ITK (CPI) impaired development of allergic lung inflammation by impairing selective expansion of Th17 lineages, impairing IL17 production from Th17 cells and reducing IL17 mediated neutrophilic infiltration. In 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.
Taurinylation of mt-tRNAs guides mitochondrial protein synthesis

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Mitochondria play a crucial role in various cellular activities, including heme and steroid synthesis, the storage of intracellular calcium ions, the regulation of cellular proliferation and differentiation, and the production of over 90% of the energy used by mammals. Despite the centrality of mitochondria to cellular functions, we know little about how mitochondria regulate its own protein synthesis. Mechanistic understanding of mitochondrial translation will help dissect the regulatory mechanisms underlying mitochondrial biology, as well as development of various cardiovascular diseases. Here we address this complex biological problem in a systematic manner, by coupling high-resolution mitochondrial ribosome profiling (mito-ribo-seq) and quantitative mapping of mitochondrial tRNA (mt-tRNA) modifications to study mitochondrial translation control. Mutations in mitochondrial DNA (mtDNA) and impaired oxidative phosphorylation have been implicated in myocardial infarction, cardiomyopathy, and heart failure. mtDNA encode a set of 13 genes for protein production, as well as a set of tRNA. For mitochondrial protein synthesis several mt-tRNAs contain \(\tau\text{m}5\text{U} \) (5-taurinomethyl) and \(\tau\text{m}5\text{s}2\text{U} \) (5-taurinomethyl-2-thio) modifications, which are critical for decoding their cognate codons. This modification has solely been discovered on mt-tRNAs, and is the only case where taurine serves as a biological component. The absence of these modifications have been observed in mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) patients carrying the A3243G mutation in mt-tRNAlue and in myoclonic epilepsy with ragged red fibers (MERRF) patients carrying the A8344G mutation in mt-tRNAlys. However, lack of in-vivo studies on taurine depletion have prevented mechanistic insight into this important physiological phenomenon. Based on current understanding of tRNA modifications, and their role in fine-tuning of protein translation we hypothesized that the presence of taurine is essential for accurate maintenance of the mitochondrial protein synthesis machinery and overall mitochondrial function. Taurine is solely produced through the conversion of cysteine by cysteine dioxygenase (CDO). We have obtained a CDO null mouse which exhibits ablated taurine levels, accumulation of acylcarnitines, and elevated plasma hydrogen sulfide. We created CDO KO mouse embryonic fibroblasts (MEFs) lacking intracellular taurine for functional analysis. Using this cell line we found lack of taurine leads to reduced mitochondrial membrane potential, impaired oxygen consumption rate, and ablated Ca\textsuperscript{2+} uptake. Radiolabeling and mito-ribo-seq experiments show that CDO KO MEFs have impaired mitochondrial translation compared to WT. Interestingly, these results can be rescued with the administration of taurine. Together, this data points towards the importance of taurine in maintaining the proper function of the mitochondria and in modifying mt-tRNAs in order to regulate mitochondrial protein synthesis.
Fibrolamellar carcinoma (FLC) is a rare form of liver cancer that disproportionately affects children and young adults, usually presents at advanced stages with no known risk factors or serum biomarkers, and exhibits high intrinsic drug resistance. Currently, no established therapies exist for this devastating disease. Recently, the signature genetic event of FLC was identified as a ~400kb heterozygous deletion on chromosome 19 resulting in the DNAJB1-PRKACA (DP) fusion gene, which occurs in over 80% of FLC patients. Two different mouse models demonstrate that the DP fusion is sufficient for liver tumor formation. Also, several prior genome-scale studies have identified hundreds of dysregulated coding- and non-coding genes in FLC tumors. However, these efforts have yet to elucidate a therapeutic strategy for FLC patients.

Super enhancers are dense clusters of highly active DNA regulatory elements that tend to be nearby genes that are critical for key tumor phenotypes. Using chromatin run-on sequencing (ChRO-seq) on primary and metastatic FLC tumor samples as well as non-malignant liver tissue, we identified 141 FLC-specific super enhancers. Moreover, we determined that the genes associated with these FLC-specific super enhancers are most strongly enriched in MAPK signaling and also associated with multi-drug resistance. Using three different FLC cell models, we found that several of these genes, notably SLC16A14, CA12 and LINC00473, are very strongly aberrantly elevated in response to DP activity. Treatment of FLC cell models with inhibitors of CA12 or SLC16A14 reduced cell viability independently and/or significantly enhanced the effect of the MAPK pathway inhibitor cobimetinib.

Overall, these findings highlight new molecular targets for drug development as well as novel drug combination approaches. This study also identified candidate onco-non-coding RNAs, such as LINC00473, which merit future detailed functional studies to investigate their role in FLC pathology.
Tick Blood Meal Analysis for Host Identification using 16S rRNA-based PCR and Illumina Sequencing

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The hematophagous behavior of vectors allows for host identification through the analysis of DNA contained in their blood meals. Molecular methods have been widely used for this purpose due to their high specificity and sensitivity, however, current approaches have showed difficulties to detect DNA when present in low concentrations in ticks, particularly in nymphs, and are not sufficiently sensitive in determining host species. The aim of this study was to develop a novel molecular approach capable of overcoming these issues and with the potential to identify multiple hosts in tick blood meals. To achieve this, DNA was extracted from eight Ixodes scapularis adult ticks collected from Acadia National Park (ANP), Long Island, NY (LI) and the Hudson Valley region of NY (HV). One lung sample from a cow and two ticks collected from horses were included as controls for the entire molecular analysis. PCR amplifications were performed with primers targeting a small region of the mitochondrial 16SrRNA gene. A tagmented library was prepared with the Nextera XT DNA Library Preparation Kit and run on a MiSeq sequencer. Sequences were analyzed in QIIME2 using VSEARCH consensus classification method with a custom-built database of vertebrate 16S sequences. Features obtained from the tick collected in the ANP belonged to the Cervidae, Canidae, and Bovidae groups. Best hits in BLAST were Odocoileus virginianus, Canis lupus, and Bos taurus. Ticks from LI and HV presented features associated with the Bovidae, Felidae, Canidae, Fringillidae, and Hominidae families. Best hits belonged to B. taurus, Felis catus, C. lupus, Haemorhous mexicanus, and Homo sapiens. Unassigned features were detected in most of the samples, all outside the expected amplicon size range. Preliminary findings provide strong evidence for the use of this method to successfully identify DNA from multiple animal hosts in tick blood meals.
Veterinarians’ perceptions about antibiotic use and resistance in dairy cattle: an international survey

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Veterinarians have a crucial role in promoting farm management measures to reduce the risk of antibiotic resistance from emerging on dairy farms, as well as educating their clients regarding the appropriate and responsible use of antibiotics. Our objective was to elucidate perceptions, attitudes, and concerns of dairy veterinarians regarding antibiotic misuse or overuse in the emergence of antibiotic resistance in dairy farming. A questionnaire survey was developed and distributed via email to veterinarians worldwide and provided at an international conference about bovine mastitis in 2018. A total of 71 veterinarians, representing 21 countries including USA, participated in the survey. Collected information included three outcomes of interest regarding veterinarians’ concerns about the emergence of antibiotic resistant infections in dairy cattle, farm workers, and the general public. The considered explanatory variables were: veterinarian’s length of experiences serving dairy farms, profit from antibiotic sale, communication with farmers, and perceptions about antibiotic use. Descriptive analyses indicated that nearly half of their clients overused or inappropriately used antibiotics. Logistic regression revealed that veterinarians were concerned about the emergence of antibiotic resistant infections on the farms they serve when they also had fewer years of experience serving dairy farms, thought that better adherence to labelling on drug packaging is an important but difficult to implement strategy to reduce client’s antibiotic use, and agreed that misuse of antibiotics contributes to the emergence of antibiotic resistance on farms. Veterinarians unconcerned about antibiotic resistance on clients’ farms tended to make more profit from antibiotic sale. These preliminary findings are expected to aid development of strategies to optimize antibiotic use in dairy farming and educate farmers and veterinarians about the appropriate and responsible use of antibiotics.
Development of a laser scalpel neurosurgical approach to treat focal neocortical epilepsy

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One out of twenty-six people will develop epilepsy within their lifetime. Focal epilepsy is characterized by seizures that initiate at one location and propagate throughout the brain. Medical management fails in about 45% of focal epilepsy cases and resective surgery remains the only alternative, but often leaves patients with severe neurologic deficits. It has been hypothesized that making less invasive incisions to severe lateral connections that seizures propagate along while maintaining vertical connections in the cortex would preserve most normal brain function while blocking seizure propagation. Tissue ablation by tightly-focused femtosecond laser pulses provides a “laser scalpel” that can make subsurface microincisions in the cortex without damaging the overlying tissue. Previously, we have shown that laser incisions in layers II-IV of rodent cortex can block acutely induced seizure propagation in 35% of rats with a 63% reduction in seizure frequency in the remaining animals. We are working on characterizing the long-term efficiency of laser ablation on blocking iron chloride induced chronic focal neocortical seizure propagation. This model develops seizures after about two weeks and has an average of 5 to 15 seizures an hour. Animals without injections show no seizures. Cuts localized to layers II-IV are minimized to an average width of 55 µm and are on average 85% complete. During imaging blood flow remained intact within the cut border. Chronic seizure propagation will be compared between animals with and without cuts to determine changes in seizure propagation. By mainly severing lateral connections we anticipate blocking seizure propagation while maintaining vertical connections to preserve cortical function.

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The left-right determining transcription factor Pitx2 regulates intestinal lymphatic development and function

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The dorsal mesentery (DM) is the major conduit for blood and lymphatic vessels as well as precursor cells reservoir in the gut. The mechanisms underlying their morphogenesis are challenging to study and remain mostly unknown. We recently discovered that arteriogenesis and lymphatic development in the DM begins during gut rotation and proceeds strictly on the left side, dependent on the left-right (LR) transcription factor Pitx2. Pitx2 is expressed in the left DM where it initiates gut rotation and patterns arteries. Pitx2⁻/⁻ mice fail to initiate gut lymphangiogenesis and die at E14.5. We obtained Pitx2ASE mice, which survive for 2 days but fail to properly express left-sided Pitx2. Interestingly, selective depletion of intestinal lymphatic endothelial precursor cells was observed in this Pitx2 deficient mouse model; loss of the asymmetric Pitx2 and Pitx2-dependent lymphatic precursor cells leads to disrupted lacteal formation, intestinal lymphangiectasia, and abnormal lipid transport function. Lipid tracer feeding in neonatal mice suggests that dietary long-chain fatty acid transport is re-routed in Pitx2 mutant mice, with aberrant liver uptake leading to diet-induced fatty liver disease. These data collectively suggest the functional importance in organ specific lymphatic endothelial precursor cell heterogeneity. Importantly, we recently identified intestinal smooth muscle cells along the margins of lymphatic endothelium as Pitx2 lineage descendants suggesting that Pitx2 contributes to intestinal lymphatic development through a non-cell autonomous program. Consistent with these findings, we report the coordinated development of intestinal smooth muscle with lymphatic endothelium during gestation, suggesting a role for smooth muscle cells in supporting a niche for lymphatic endothelial cell maturation. Here we present a model where Pitx2 expression links smooth muscle development with lacteal morphogenesis, connecting early embryonic laterality pathway with the development of functional lymphatic lacteals.
How does the environment affect persistence of antimicrobial resistance on farms and could it play a role in mitigation strategies? - an in silico study

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Antimicrobial resistant (AMR) infections in humans are a global concern, and AMR bacteria may be transmitted from farm animals to people directly or via the environment. Previous field studies support that AMR bacteria can increase in population size in farm environments given sufficient nutrients, despite having a competitive disadvantage due to fitness cost. Our objective was to test the hypothesis that even in the absence of antibiotic use AMR bacteria can persist in farm environments long after animals colonized with AMR bacteria are introduced into the environment. We developed a deterministic compartmental model, based on ordinary differential equations, and solved it numerically to predict the dynamics of enteric bacteria within a group of heifers and the environment in their self-contained pen. The impact of direct transmission between animals is assumed to be negligible. The model is deterministic i.e. the role of chance in transmission events or stochastic fade-out is assumed to be negligible. Monte Carlo simulation was used to explore the impact of uncertainty in parameter values and initial conditions on model predictions. The model was run for 250 days for 100,000 iterations. Preliminary results suggest that when the death rate of enteric bacteria in the environment is low (such as when the pen is not cleaned frequently), then there can be persistence of AMR bacteria in the pen over several days to months after the animals are introduced to the pen.
Extracellular Vesicles and the Maintenance of Stemness in Embryonic Stem Cells

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Pluripotent embryonic stem cells (ESCs) reside in blastocyst-stage embryos, until the proper time when they receive signals to differentiate into the germ layers that will ultimately give rise to the entire organism. However, the mechanisms underlying the maintenance of pluripotency in ESCs remain poorly understood. Here, we show that ESCs generate extracellular vesicles (EVs), including exosomes and microvesicles (MVs), to promote stemness. Treating ESCs undergoing differentiation with exosomes and/or MVs isolated from naïve pluripotent ESCs inhibited their differentiation and maintained several stem cell characteristics, including the ability to form spheres, exhibit alkaline phosphatase activity, as well as maintain the expression of master regulators of pluripotency Oct3/4 and Nanog. These effects were dependent on the large amounts of fibronectin associated with the vesicles, and its ability to bind integrins and activate focal adhesion kinase (FAK) in the differentiating ESCs. We also determined that the pluripotency of ESCs in blastocysts were similarly maintained by ESC-derived EVs, highlighting how this unique form of intercellular communication can promote stemness.
Osteoarthritis functional assessment in the rat anterior cruciate ligament transection model


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The rat surgical anterior cruciate ligament transection (ACLT) model is commonly used to study osteoarthritis (OA) therapies. Histologic scoring is employed as a gold standard for OA assessment in rodent models; however, histology does not necessarily correlate well with clinical signs, and imaging and functional outcomes are used to evaluate OA in human patients. Evaluation of functional and pain-related outcomes in rodent PTOA models will increase their translational value. Here, we examined four functional measurements for assessing surgical ACLT: static weight-bearing (incapacitance), mechanical allodynia (vonFrey analgesiometry), motor function (rotarod), and dynamic gait analysis to determine whether these outcome measures could differentiate between naïve unoperated rats and rats undergoing surgical ACLT with either sham or synthetic biomimetic boundary lubricant (sBBL) injections.

Histopathological changes 20 weeks after ACLT surgery were assessed using H&E and SafO/Fast Green-stained sections obtained from the medial tibial plateau using the modified Mankin scoring system. Histologic grading revealed cartilage fibrillation and matrix loss in saline and sBBL-injected ACLT joints as compared to naïve joints, with a median total Mankin grade of 0 for naïve controls, 4 for saline-treated ACLT joints and 5 for sBBL-treated ACLT joints. Functional outcomes were impaired immediately post-ACLT for all measurements with the exception of rotarod testing; however, most functional parameters recovered to baseline after week 6. Of the four functional measurements, static weight-bearing was the most reliable and sensitive method for detecting differences in the early post-operative period. Although von Frey testing showed a similar pattern to static weight-bearing, data was more variable. Left print length, left toe spread, and rib height in the dynamic gait analysis showed similar decreased performance post-operatively and complete recovery in the later stage. Here, we demonstrate that, despite histological evidence of OA, group differences in functional parameters were predominantly restricted to early, post-operative changes in the rat surgical ACLT model. In rats, ACLT alone may be insufficient to induce functional or gait changes that mimic chronic OA in humans, at least over the course of a 20-week study duration.