Long Non-coding RNAs Symposium

From Basic Mechanism to Human Disease

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Abstract Book

National Heart, Lung, and Blood Institute

2021 NHLBI LONG NON-CODING (LNC) RNAS SYMPOSIUM:

From Basic Mechanism to Human Disease

ABSTRACT BOOK

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Abstracts

P1. FUNCTIONAL CLASSIFICATION OF LONG NONCODING RNAS BY K-MER CONTENT

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LncRNAs play essential roles in health and disease but their functions remain difficult to predict from their sequence. In contrast to proteins, lncRNAs with similar function often lack linear sequence similarity; thus, the identification of function in one lncRNA rarely informs the identification of function in others. We developed an alignment-free approach called SEEKR that utilizes sequence substrings called *k*-mers to compare RNA sequence. SEEKR can identify similarities between lncRNAs that are invisible by linear sequence alignment, including similarities in protein-binding potential, subcellular localization, and biological activity (see PMIDs 30224646, 31097619, 33326069). Our data support the emerging notion that different lncRNAs can encode related functions through RNA elements that lack linear-sequence similarity yet are enriched in similar subsets of structural or protein-binding motifs. We are in the process of expanding the functionality of SEEKR, including developing and vetting approaches to assess the significance of SEEKR-defined similarity and to scan transcriptomes for lncRNAs or lncRNA domains that harbor related functions. The long-term goal of this research is to establish paradigms and computational frameworks to identify recurrent relationships between sequence and regulatory function in any set of lncRNAs.

P2. ELUCIDATING THE ROLE OF MALAT1 IN LUNG ADENOCARCINOMA

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Lung cancer is the leading cause of cancer-related deaths worldwide. Growing evidence suggest that several long noncoding RNAs (lncRNAs) are aberrantly expressed in lung adenocarcinoma (ADC) and show significant correlation with patient prognosis, suggesting the potential use of lncRNAs as diagnostic markers and therapeutic targets. In particular, the lncRNA MALAT1 (Metastasis Associated Lung Adenocarcinoma Transcript 1) is frequently elevated in lung tumors and highly predictive of metastatic incidence and poor survival. However, these correlative observations do not reveal whether the aberrantly expressed lncRNA is a driver of tumorigenesis or a passive target of the global epigenetic reprogramming in human cancer. To elucidate the role of MALAT1 in lung ADC, we have developed and validated a CRISPR/Cas9-based approach to modulate MALAT1 expression levels in patient-derived cell lines and animal models of lung adenocarcinoma. Strikingly, we find that transcriptional activation of MALAT1 is sufficient to increase cellular migration i*n vitro* and to dramatically advance tumor progression *in vivo* in the autochthonous K-ras/p53 mouse model of lung adenocarcinoma. In contrast, transcriptional downregulation of MALAT1 suppresses cellular migration. Interestingly, changes in MALAT1 levels do not affect cellular proliferation or apoptosis, suggesting a selective role for MALAT1 in regulating cellular migration. Lastly, gene expression profiling of cells with aberrant MALAT1 expression highlights potential mediators of the pro-metastatic function of MALAT1. These studies advance our understanding of the involvement of MALAT1 in cancer development *in vivo* and offer valuable knowledge for novel strategies that expand the druggable space in cancer by harnessing lncRNAs for therapeutic applications.

P3. MITOCHONDRIA-ENRICHED LONG NONCODING RNAS: A NEW CLASS OF EPIGENETIC PLAYERS IN COORDINATING METABOLIC REPROGRAMMING IN TUMORS

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Background. Metabolic reprogramming is a major hallmark of cancer cells. Due to the Warburg effect, tumor cells preferentially bypass the tricarboxylic acid cycle and switch to glycolytic energy metabolism despite available oxygen. Currently, we know very little about the mechanisms underlying this metabolic switch in cancer. Long noncoding RNAs (lncRNAs) are key epigenetic regulators of metabolism. A better understanding of the role of these lncRNAs in cancer metabolism may reveal new therapeutic targets for mitochondria-targeted anticancer drugs.

Methods. To characterize the role of lncRNAs in cancer metabolic reprogramming, we isolated mitochondria from hepatoma HepG2 cells and normal hepatic HL7702 cells. RNA sequencing (RNA-seq) was performed to identify mitochondrial RNA components that are differentially associated with mitochondria between hepatoma normal hepatic cells. The role of mitochondrial-associated lncRNAs was examined by shRNA knockdown and LwaCas13a-BN-MLS mitochondrial RNA targeting. Mitochondrial biogenesis and energetics were examined by mitochondrial copy number, ATP synthesis, real-time oxygen consumption rate (OCR), the extracellular acidification rate (ECAR), and mitochondrial-derived reactive oxygen species (mROS).

Results. Using RNA-seq, we identified a total of 246 RNAs that were differentially expressed between HepG2 and HL7702 cells. Among them, the nuclear-encoded lncRNA *MALAT1* (metastasis-associated lung adenocarcinoma transcript 1) was aberrantly enriched in the mitochondria of hepatoma cells. Notably, *MALAT1* interacted with multiple loci on the mitochondrial DNA genome, including D-loop, COX2, ND3, and CYTB genes. Knockdown of this lncRNA induced alterations in the CpG methylation of mitochondrial DNA and in mitochondrial transcriptomes. In cells

in which *MALAT1*-was knocked down, we found multiple abnormalities in mitochondrial function, including altered mitochondrial structure, low OXPHOS, decreased ATP production, reduced mitophagy, decreased mtDNA copy number, and activation of mitochondrial apoptosis. These alterations in mitochondrial metabolism were associated with changes in pathways involved in cell mitophagy. We further showed that the RNA-shuttling protein HuR and the mitochondria transmembrane protein MTCH2 mediated the transport of *MALAT1* in this nuclear-mitochondrial crosstalk.

Conclusion. This study provides the first evidence that the nuclear genome-encoded lncRNA *MALAT1* may function as a critical epigenetic player in the regulation of mitochondrial metabolism of hepatoma cells, laying the foundation for further clarifying the roles of lncRNAs in tumor metabolic reprogramming.

P4. FUNCTIONAL CHARACTERIZATION OF MUNC LNCRNA STRUCTURAL DOMAINS IN CONTEXT OF SKELETAL MUSCLE DIFFERENTIATION AND GENE EXPRESSION REGULATION

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Background. Although thousands of long non-coding RNAs (lncRNAs) have been already discovered, only few are functionally characterized and even fewer provide insights into molecular mechanism based on their structure. Therefore, studies of lncRNA structure and subsequent elucidation of its function and the regulatory mechanisms are urgently needed. *MUNC* lncRNA is upregulated in murine skeletal muscles and was firstly described as a facilitator of *Myod1* function in myogenesis. There are two *MUNC* isoforms: spliced and genomic, and both have promyogenic abilities. *siMUNC* reduces myoblast differentiation, and stable over-expression stimulates promyogenic RNAs. *MUNC* is also one of the conserved lncRNA and its homolog can be found in human muscles.

Methods. In this study we focus on the relationship between *MUNC* lncRNA structure and function. To analyze the functional similarities and differences between the *MUNC* two isoforms, we performed RNA-seq on myoblasts overexpressing *MUNC* spliced or genomic in proliferation and differentiation conditions. We obtained the secondary structure of both isoforms using SHAPE-MaP technique. Knowing that both *MUNC* isoforms induce the same promyogenic genes, we systematically deleted the most structurally similar regions. We tested their functional importance by overexpressing the mutated constructs in murine myoblasts and performing differentiation assays. To find out which domains are required for binding specific genomic sites, we did *MUNC* Chromatin Isolation by RNA Purification followed by qPCR.

Results. We showed that both *MUNC* transcripts can regulate different sets of genes that are important for muscle structure development, RNA processing and apoptosis signaling. We found that *MUNC* spliced and genomic have six common structural

domains and eight common proteins protected sites. We have identified domains which are required for induction of *Myod1*, *Myogenin* and *Myh3.* We have also found out a domain which is necessary for *Myod1* stimulation, but not *Myogenin*, proving again that *MUNC* can act both *in cis* and *in trans*. We discovered which *MUNC* domains are responsible for binding specific genomic sites and regulation of expression of adjoining genes.

Conclusion. We determined the secondary structure of *MUNC* and found that specific structural domains are critical in the process of skeletal muscle differentiation. Moreover, we characterized structural features facilitate *MUNC* binding to specific genomic sites and protein interactors. Using the data about *MUNC* functional domains, protein binding partners and genomic sites interactions we can now better characterize this lncRNA and describe its mechanism of function.

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P5. PAN-CANCER ANALYSIS REVEALS TAP63-REGULATED ONCOGENIC LNCRNAS THAT PROMOTE CANCER PROGRESSION THROUGH AKT ACTIVATION

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Background. Metastatic breast cancer is characterized by *TP53* mutations and activation of the PI3K/AKT pathway, two events crucial for the progression of this disease. Mutations in *TP53* cause the inhibition of the p53 family member and p63 isoform, TAp63, which we have previously demonstrated to be a crucial tumor and metastasis suppressor. Indeed, loss of *TAp63* in mice transforms mammary epithelial cells (MECs) into tumor initiating cells, ultimately leading to mammary adenocarcinomas.

Methods. A mouse-human cross species analysis was performed to identify long non-coding RNAs (lncRNAs) that were both regulated by TAp63 in MECs and differentially expressed in a human model of breast cancer progression. Orthotopic xenograft models and tail vein injections were carried out to assess the tumorigenic and metastatic role of the identified lncRNAs. In situ hybridization for these lncRNAs was conducted in tissue microarrays (TMAs) of multiple cancer types to explore possible connections between the levels of these lncRNAs and clinic features of human cancer patients.

Results. Using multiple mouse models of aggressive cancers combined with investigation of human TMAs and TCGA datasets, we unveiled the pivotal role of two TAp63 regulated lncRNAs, *TROLL-2* and *TROLL-3*, across a broad variety of cancer types. The levels of these two lncRNAs are elevated in tumors harboring *TP53* mutations and their downregulation in mouse models of aggressive cancers resulted in a marked suppression of tumor and metastasis formation. The characterization of these mouse models and a pan-cancer analysis including 723 human biopsies revealed that these two lncRNAs are markers of cancer progression.

Specifically, they regulate the nuclear to cytoplasmic translocation of their effector protein, WDR26, which in turn induces the activation of the PI3K/AKT pathway to promote tumor progression. In line with this, we found that high expression of *TROLL-2* and *TROLL-3*correlates with WDR26 cytoplasmic location and increased pAKT levels in a wide variety of human metastatic cancers, including breast, ovarian, colon, lung adenocarcinoma, lung squamous cell carcinoma, and melanoma.

Conclusion. Our data provide preclinical rationale for the implementation of these lncRNAs and WDR26 as novel therapeutic targets for the treatment of human tumors dependent upon *TP53* mutations and/or activation of the PI3K/AKT pathway.

P6. A RIG-I DEPENDENT TISSUE SPECIFIC ANGIOGENIC PROGRAM MAINTAINS ENDOTHELIAL FUNCTION

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Activation of nucleic acid sensors in endothelial cells (ECs) has been shown to drive inflammation in cancer atherosclerosis and obesity. Endogenous non-coding RNAs as well as mis-spliced mRNAs can trigger nucleic acid sensors in the cytoplasm. We previously showed that enhancing cytosolic DNA sensing by inhibiting three prime exonuclease 1 (TREX1) in ECs led to EC dysfunction. Here we show how activation of a cytosolic RNA sensor, Retinoic acid Induced Gene 1 (RIG-I) diminishes EC survival, angiogenesis and triggers gene expression programs that affect angiogenesis, thrombosis and inflammation. We evaluated a potent 79 nucleotide hairpin loop RIG-I agonist derived from the H1N1 influenza virus. This agonist significantly decreased proliferation and survival in cultured ECs in vitro and in vivo. We confirmed that this agonist induced gene expression programs characteristic of EC injury including cell death, oxidative stress, inflammation and type I interferon signature genes. We observed that RIG-I$^{-/-}$ mice have aberrant angiogenic responses. Interestingly, we found that RIG-I$^{-/-}$ mice have distinct tissue specific angiogenic signatures. Using a combination of cultured ECs and a RIG-/- mouse, we have discovered a RIG-I dependent 6 gene angiogenic signature that affects angiogenesis, inflammation and coagulation. We propose that activation of RIG-I pathway in different vascular beds can drive distinct pathological sequelae associated with diseases ranging from viral infections to cancer.

P7. LONG NONCODING RNAS IN CARDIAC FIBROBLASTS: MECHANISMS OF EPIGENETIC REGULATION

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Background. Cardiac fibrosis is driven by activated fibroblasts and is observed in heart failure. Activated fibroblasts have dysregulated transcriptomes in disease, and previous investigations have characterized roles for long noncoding RNAs (lncRNAs) in regulating expression of specific protein-coding genes involved in cardiac fibroblast activation and other cardiac pathologies, suggesting an important role for noncoding genomic features in the heart. Recently, others have shown that the binding landscape of the chromatin reader BRD4 is dynamic in cardiac fibroblasts after activation with TGFb, which partially explains widespread transcriptomic shifts observed with activation. JQ1, a small molecule BRD4 inhibitor, blunts the TGFb response in cardiac fibroblasts *and* abrogates BRD4 binding at a specific promoter and its enhancer elements normally turned on with TGFb. However, we lack a genome-wide picture of lncRNA transcriptional changes when TGFb-induced cardiac fibroblasts are then inhibited by JQ1. Because of their spatial proximity, we hypothesized that lncRNA expression may regulate expression of nearby mRNA.

Methods. Using RNA-seq data from isolated murine cardiac fibroblasts treated with DMSO (vehicle), JQ1, TGFb, or both TGFb and JQ1 (n=3/group), we identified differentially expressed (padj<0.05) lncRNAs and their nearest protein-coding genes.

Results. Six comparisons revealed differentially expressed lncRNAs (annotated as treatment/control): 343 in JQ1/DMSO, 39 in TGFb/DMSO, 331 in TGFb-JQ1/DMSO, 392 in TGFb/JQ1, 123 in TGFb-JQ1/JQ1, and 304 in TGFb-JQ1/TGFb. A comparison of log2FoldChange between lncRNAs and their closest protein-coding genes revealed similar trends: when lncRNA genes were upregulated, there tended to be corresponding upregulation of the closest mRNA (the same could be said for those

that were downregulated) while there were fewer instances of upregulated lncRNAs corresponding to downregulated mRNAs and *vice versa*.

Conclusion. We observed the most differentially expressed lncRNAs in the TGFb/JQ1 comparison, highlighting a powerful effect of JQ1 on noncoding RNA abundance. Taken together, our data support a correlation between lncRNA transcription and the transcription of nearby mRNA genes. Further study of the actions of lncRNAs and their transcription will reveal the mechanisms by which these noncoding transcripts regulate mRNA abundance and in turn contribute to our understanding of the role of cardiac fibroblasts in heart failure.

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P8. TARGETED KILLING OF HIV-1 INFECTED MACROPHAGES THROUGH MODULATION OF THE LNCRNA SAF

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Background. Long non-coding RNAs (lncRNA) are emerging as crucial regulators in a variety of physiological and pathophysiological pathways; and therefore are appealing as important therapeutic targets for many diseases including HIV-1. Knowledge about the role of lncRNAs in HIV-1 infection is still incipient, particularly in macrophages, which are notably resistant to the viral cytopathic death and constitute a long-lived cellular reservoir of HIV-1. Here, we have examined and identified the role of a lncRNA in regulation of apoptosis in HIV-1 infection of macrophages and determined the effects of modulation of the lncRNA on the cellular fate of the virus-infected cells.

Methods. Human monocytes were cultured for 7 days to differentiate into macrophages (HMDM) that were then infected with a mCherry-expressing NL4-3 (BaL env) HIV-1 virus. Alveolar macrophages (AM) were obtained from the lungs of HIV-1 infected individuals by bronchoalveolar lavage. Expression of ninety well-characterized lncRNAs were examined by qRT-PCR in three flow-sorted cell populations: virus infected, virus exposed but uninfected bystander and virus non-exposed HMDMs. Additionally, siRNA was used for targeted down-regulation of specific lncRNA and its effect on apoptosis of HIV-1 infected HMDMs was determined by flow cytometric detection of active caspase-3 in the cells.

Results. Expression of the anti-apoptotic lncRNA SAF (FAS-AS1) was found to be significantly up-regulated in HIV-1 infected HMDMs compared to both the bystander and control HMDMs. Similar elevated level of SAF expression was also observed in HIV-1-positive alveolar macrophages (AM) obtained from the lungs of HIV-1 infected individuals by bronchoalveolar lavage. Furthermore, down-regulation of SAF lncRNA with siRNA led to a significant increase in active caspase-3 level in HIV-1 infected HMDMs. This siSAF-mediated activation of apoptotic caspases and subsequent cell death occurred specifically in HIV-1 infected HMDMs but not in

bystander or virus non-exposed cells. The targeted induction of apoptosis in virus-infected cells also resulted in a significant reduction in overall viral load in the HIV-1 infected macrophage culture.

Conclusion. Our results show that the lncRNA SAF plays a key role in cell survival of HIV-1 infected macrophages and siRNA-mediated modulation of this lncRNA offers a potential new therapeutic approach to specifically eliminate virus-infected macrophage reservoirs.

P9. NORAD-INDUCED PHASE SEPARATION OF PUMILIO PROTEINS IS REQUIRED FOR GENOME MAINTENANCE

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Long noncoding RNAs (lncRNAs) are a heterogenous class of non-protein-coding transcripts that have frequently been proposed to bind and regulate the activity of RNA binding proteins (RBPs). In most cases, however, lncRNA transcripts are greatly outnumbered by their associated RBPs or by other transcripts that can bind the relevant RBPs. Thus, the mechanisms through which lncRNAs are able to efficiently recruit RBPs and regulate their activity have remained unclear. This problem is exemplified by *NORAD*, a highly conserved and abundant cytoplasmic lncRNA that is required for genome stability in mammals. *NORAD* functions by binding to and negatively regulating PUMILIO (PUM) RBPs. Loss of *NORAD* results in PUM hyperactivity, leading to the repression of PUM target messenger RNAs (mRNAs) which include important regulators of mitosis, DNA replication and DNA repair, ultimately resulting in a dramatic genomic instability phenotype in *NORAD*-deficient cells and mouse tissues. Despite compelling genetic evidence implicating *NORAD* as a negative regulator of PUM, it remains enigmatic how this lncRNA is able to efficiently regulate these RBPs given that *NORAD* is greatly outnumbered by other PUM target transcripts. For example, in HCT116 cells, a model cell line in which *NORAD* has been shown to regulate PUM and genomic stability, *NORAD* is expressed at ~400 copies per cell. With 18 PUM binding sites per transcript, *NORAD* provides only ~7200 PUM binding sites to compete against ~130,000-325,000 PUM binding sites in expressed mRNAs. Thus, simple competitive titration of PUM by *NORAD* is unlikely to meaningfully impact the pool of free PUM that is available to regulate target mRNAs.

Here we show that *NORAD* is able to out-compete thousands of other PUM-binding transcripts to bind and inhibit PUM activity by nucleating the formation of phase-separated PUM condensates, termed *NORAD*-PUMILIO (NP) bodies. Dual mechanisms of PUM recruitment, involving multivalent PUM-*NORAD* and PUM-PUM

interactions, enable *NORAD* to competitively sequester a super-stoichiometric amount of PUM in these condensates. Importantly, disruption of *NORAD*-driven PUM phase separation leads to PUM hyperactivity and genome instability that is rescued by synthetic RNAs that induce PUM condensate formation. These results reveal a new mechanism of RBP regulation by RNA-driven phase separation and uncover an essential role for this process in genome maintenance. The repetitive sequence architecture of *NORAD* and other lncRNAs suggests that phase separation may be a widely used mechanism of lncRNA-mediated regulation.

P10. LNCRNA PTCHD1-AS: A NOVEL HIGH FUNCTIONING ASD MOUSE MODEL

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Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental disorder characterized by social communication deficits and the display of restrictive, repetitive behaviors. Early studies evaluating the contribution of copy number variants (CNVs) have implicated the *PTCHD1* locus in ASD development. Located at Xp22.11, it encompasses genes *PTCHD1, DDX53* and long non-coding RNA (lncRNA) *PTCHD1-AS*, and is regarded as a highly-penetrant risk locus in males, contributing to an estimated ~1% of ASD and intellectual disability (ID) cases. Evaluating both published and unpublished data from individuals with a constellation of neurodevelopmental phenotypes to assess the correlation between ASD and variants disrupting this locus suggests that, in fact, it is the *PTCHD1-AS,* which is underlying the association with ASD. Given the high penetrance of ASD linked to *PTCHD1-AS* microdeletions, we have initiated a characterization and functional study to elucidate its role in the etiology and expression of autism in a developmental model. Guided closely by the human phenotype data, we are assaying for behavioral and cellular effects in Ptchd1-as mutant mice to look at the transcriptional and translational consequences of a deletion in the critical region associated with high functioning autism.

P11. RELOT IS A NOVEL LNCRNA REGULATOR OF ALLOGENEIC T CELL FUNCTION AND T CELL RECEPTOR SIGNAL TRANSDUCTION

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Allogeneic T cell responses drive graft rejection following solid organ allograft transplantation and acute graft-versus-host disease (GVHD) following hematopoietic stem cell transplantation (HSCT). However, the mechanisms regulating allogeneic T cells are incompletely understood. Long non-coding RNAs (lncRNA) are emerging tissue-specific modifiers of immune responses, but their role in alloimmune T cell responses has not been defined.

To identify lncRNAs involved in regulation of allogeneic T cells after clinical HSCT, we recently performed RNA-seq on T cells from three different groups of well-annotated HSCT recipients that differed in their degree of histocompatibility mismatch. We observed greater differential expression of lncRNAs in donor T cells isolated from allogeneic relative to autologous HSCT recipients. These data suggested lncRNAs regulate alloimmune T cells. Among the 13 differentially expressed lncRNAs in T cells following clinical allogeneic HSCT, we nominated the novel lncRNA, *LINC00402,* for further characterization for the following reasons: 1) its expression was enriched in T cells 88.3 fold relative to all other cell types analyzed in the FANTOM5 database, 2) it was predicted to be conserved among mammals, a feature associated with functional lncRNAs, and 3) it was predicted to

lack protein coding potential. Here, we validated *LINC00402's* differential expression, function, and molecular mechanisms.

LINC00402 was differentially expressed in allogeneic T cells in two HSC and one cardiac transplantation patient cohort (pre-transplant fold change 1.29 vs. 0.29 post-transplant, p=0.002). Its expression was rapidly down-regulated upon T cell activation and was preserved by inhibiting T cell activation with tacrolimus, cyclosporine, mycophenolic acid, or rapamycin. Functionally, *LINC00402* augmented T cell proliferation following an allogeneic stimulus (CRISPR/Cas9-mediated *LINC00402*-depleted cells proliferated 58% of controls, p=0.02) but not to a nominal ovalbumin peptide antigen or poly-clonal anti-CD3/CD28 stimulus. Taken together, these data suggested that *LINC00402* expression was down-regulated via a negative feedback loop following activation and that *LINC00402* promoted allogeneic T cell function.

Prior studies demonstrated that sub-optimal T cell inhibition correlated with increased development of GVHD. Therefore, we predicted lower *LINC00402* expression, representing presumed greater T cell activation, would correlate with increased development of GVHD. Consistent with this, *LINC00402* expression in T cells was lower in allogeneic HSCT recipients who subsequently developed acute GVHD relative to those who did not (*LINC00402* relative change of 2.62 vs. 0.84 for those who did not and did subsequently develop GVHD, p=0.0092).

Long non-coding RNA molecular mechanisms often correlate with their subcellular localization. *LINC00402* expression was similar in the cytoplasm and nucleus. Consistent with its presence in the cytoplasm, *LINC00402* enhanced MEK1/2 activation, ERK1/2 activation, increased c-FOS nuclear accumulation, and augmented the expression of IL-2 and *Egr-1* following T cell receptor engagement. These data suggested that *LINC00402* enhanced a MEK-ERK-c-FOS pathway following T cell activation.

Altogether, our studies identified *LINC00402* as a novel, conserved regulator of allogeneic T cell function; therefore, we propose re-naming *LINC00402* to "regulatory long non-coding RNA of T cells" (*ReLoT*). Because of its T cell specific expression and impact on allogeneic T cell responses, targeting *LINC00402* may improve outcomes after allogeneic HSC and solid organ transplantation.

P12. RNA METHYLATION OF LNCRNA LOC339803 DICTATES CELL-TYPE DEPENDENT LOCALIZATION AND CONFERS TISSUE-SPECIFIC RISK TO AUTOIMMUNITY.

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Background. *LOC339803* is a lncRNA with unknown function located on an autoimmunity associated region. Interestingly the allele that increases the risk for intestinal inflammatory diseases (*G) confers protection for multiple sclerosis (MS). One of the disease-associated SNPs (rs11498) within an intron of the lncRNA, is close to an m^6A methylation motif, so we hypothesized that SNP-related methylation changes could affect lncRNA function, conferring risk or protection to immune diseases.

Methods. HCT15 intestinal and SH-SY5Y neuron-derived cell lines heterozygous for rs11498 were used for *in vitro* experiments. Immunoprecipitation of *in vitro* transcribed biotinylated *LOC339803* followed by mass spectrometry (RIP-MS) in each cell type was used to detect lncRNA-protein interactions. Intestinal biopsies from control and celiac disease patients and myelinated versus demyelinated grey matter samples from progressive MS patients were used for the quantification of lncRNA expression.

Results. To confirm whether the allele genotype is influencing m^6A methylation levels of the lncRNA, we performed an allele-specific meRIP in intestinal and neuron-derived cell lines. We observed that the G allele is preferentially methylated independently of the cell origin. Assessment of the subcellular location showed that *LOC339803* is primarily nuclear in intestinal cells but mainly cytoplasmic in neurons, pointing to a tissue-dependent function.

It is widely known that m^6A methylated RNAs are identified by diverse m^6A readers which influence RNA fate. Terminus specific binding to YTHDC1 reader has been related to differential subcellular localization and function of methylated RNAs. RIP-MS showed that chromatin associated transcription repressor proteins, such as HDAC1 or TRIM28, are enriched in the IP of intestinal cells. However, in the neuron-derived cells, RIP-MS showed that *LOC339803* interacts with the nuclear export proteins SRSF3 and NXF1. Interestingly, these *LOC339803*-interacting proteins have been described to also bind YTHDC1.

Moreover, we observed that in intestinal cells the nuclear *LOC339803* forms a m^6A related repressive complex that binds to the chromatin. The complex recruits HDAC1 to the adjacent *COMMD1* gene promoter, inducing an allele-dependent downregulation of this gene. Subsequently, *COMMD1* repression induces NFkB activation and increased intestinal disease related cytokine expression. On the other side, we found that the neuronal cytoplasmic *LOC339803* regulates HK2 protein levels, and influences mitochondria independent ATP levels.

To confirm the involvement of *LOC339803* in autoimmune disorders, we next quantified the lncRNA expression in human samples. In line with the *in vitro* results, celiac disease patients, with intestinal inflammation and increased NFkB and cytokine expression, show higher *LOC339803* expression levels when compared with non-celiac controls. Moreover, we observed that in MS patients, in which ATP levels have been described to be decreased, *LOC339803* expression is downregulated in the demyelinated gray matter samples.

Conclusion. Our results suggest that *LOC339803* has a tissue-dependent function that could be related to the development of intestinal inflammatory disorders and multiple sclerosis. Moreover, the lncRNA function seems to be affected by allele-specific m^6A methylation and subcellular location, which is probably mediated by the differential binding of both alleles to the YTHDC1 protein in each cell type.

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P13. CYTOSOLIC AND NUCLEAR LNCRNA HL6 COORDINATE WITH MICRORNA-1 AND PRC2 EPIGENETIC COMPLEX TO ORCHESTRATE HUMAN CARDIOGENESIS

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Background. Over the past decades, studies of heart development were focused on conserved gene regulatory mechanisms, including transcription factors, microRNAs and epigenetic complexes, which controll steps of cardiogenesis in multiple species from drosophila to mouse. However, compared to rodents,human embryos exhibit unique dynamics and properties of cardiogenesis. These species differences suggest the existence of novel human-specific genetic regulatory programs underlying early stage human heart development. Recent evidences indicate that long non-coding RNAs (lncRNAs), which are >200 bp transcripts with limited coding potential, play species-specific vital rolesin mammalian organogenesis and diseases.Due to lack of in vivo model, study of human-specific mechanisms in early cardiogenesis still remains as a challenge.

Methods. We developed an in vitro system to model human cardiogenesis by using human pluripotent stem cells (hPSCs) (Nature. 2008. 453: 524-528). This stem cell model follows each step of human cardiogenesis and provides a unique system for conducting mechanistic studies of human cardiogenesis. By performing mRNA-seq, we identified a novel human-specific lncRNA, and named Heart Brake LncRNA 1 (HBL1) (Dev. Cell. 2017. 42: 333-348). Two microRNA-1 binding sites on HBL1 were predicted and functionally validated via CRISPR/Cas-9 mediated gene editing. In nucleus, HBL1 interacting proteins were pulled down by biotin-labeled HBL1, followed with mass spectrometry. EMSA and RNA-IP confirmed the HBL1 interaction with PRC2 epigenetic complex. ChIP-seq was then conducted to uncover the impact of HBL1 on genome-wide PRC2 occupancy on essential cardiogenic genes in hPSCs. Finally, we identified that nuclear gene JARID2 is a new target of micRNA-1, and explored the impact of cytosolic “HBL1-microRNA-1” interaction on the nuclear “JARID2/PRC2-HBL1” complex in controlling transcription of key cardiogenic genes.

Results. HBL1 expresses in both cytoplasm and nucleus of hPSCs. Gain-of-HBL1 represses, whereas loss-of-HBL1 increases cardiomyocyte differentiation from

hPSCs. Cytosolic and nuclear fractions of HBL1 employ different mechanisms, which crosstalk to regulate cardiogenesis from hPSCs. Cytosolic HBL1modulates cardiac development from hPSCs by fine-tuning the bioactivity of microRNA-1. In hPSCs, disruption of microRNA-1 binding sites on HBL1 phenocopies HBL1deficiency. Nuclear HBL1 interacts with two PRC2 subunits, JARID2 and EED. Loss-of-JARID2 or EED phenocopies HBL1deficiency to significantly enhance cardiac differentiation from hPSCs. HBL1 deficiency disrupts genome-wide PRC2 occupancy and H3K27me3 chromatin modification on essential cardiogenic genes. Hence, HBL1-depletion significantly and broadly enhances cardiogenic gene transcription in hPSCs and during hPSC differentiation. Additionally, ChIP-seq reveals reduced PRC2-occupancy on 62 overlapped cardiogenic genes in HBL1$^{-/-}$and JARID2$^{-/-}$hPSCs, indicating both HBL1 and JARID2 are required for PRC2 deposition to set up the epigenetic state of critical cardiogenic genes in undifferentiated hPSCs. Furthermore, after cardiac development occurs, the cytosolic and nuclear fractions of HBL1 could crosstalk via a conserved “microRNA-1-JARID2” axis to modulate transcription of cardiogenic genes.

Conclusion. Overall, our findings delineate the indispensable role of human-specific HBL1 in early human cardiogenesis, and expand the mechanistic scope of lncRNA(s) that cytosolic and nuclear portions of HBL1 could coordinate differential molecular mechanisms to precisely control human heart development.

P14. A NOVEL LNCRNA DRAIR IS DOWNREGULATED IN DIABETIC MONOCYTES AND MODULATES INFLAMMATORY PHENOTYPE VIA EPIGENETIC MECHANISMS

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Background. Type 2 Diabetes Mellitus (T2D) is associated with chronic inflammation implicated in cardiovascular complications. Activation of innate immune cells (monocytes and macrophages) by high glucose and elevated free fatty acids is a key driver of prolonged inflammation. Evidence shows that long non-coding RNAs (lncRNAs) regulate inflammatory pathways in innate immune cells through epigenetic mechanisms. We examined the involvement of a previously uncharacterized lncRNA DRAIR (Diabetes regulated anti-inflammatory lncRNA) in monocyte/macrophage dysfunction and inflammation in diabetes.

Methods. RNA from $CD14^+$ blood monocytes from T2D patients and non-diabetic controls was analyzed by strand specific RNA-seq. *DRAIR* regulation and functions were determined in primary CD14+ monocytes and cultured human THP1 monocytes by RT-qPCR, knockdown with siRNAs, overexpresson using lentiviral or plasmid vectors, monocyte-endothelial cell adhesion assays, phagocytosis of *E. coli* particles, and promoter analysis with luciferase reporter assays. Epigenetic mechanisms were examined using ChIRP-seq and ChIRP-mass spectrometry, RNA-pulldown, RNA-immunoprecipitation, and ChIP assays. Mouse Drair was studied in peritoneal macrophages from T2D db/db mice and non-diabetic mice. Drair knockdown in mouse macrophages was performed using LNA-Gapmers.

Results. RNA-seq analysis revealed upregulation of inflammatory genes, but downregulation of anti-inflammatory and anti-proliferative genes in $CD14^+$ monocytes from T2D patients versus controls supporting enhanced inflammation and monocytosis. Several lncRNAs including a novel divergent lncRNA *DRAIR* and the

adjacent gene, *CPEB2* were also downregulated. High glucose and palmitic acid downregulated *DRAIR* in monocytes. Conversely, *DRAIR* expression was upregulated by macrophage differentiation and treatment with anti-inflammatory cytokines via KLF4, a key transcription factor involved in alternative activation of macrophages. *DRAIR* overexpression increased anti-inflammatory and macrophage differentiation genes but inhibited pro-inflammatory genes. Conversely, its knockdown attenuated anti-inflammatory genes, promoted monocyte-endothelial adhesion, and inhibited phagocytosis in THP1 cells. Similarly, *DRAIR* knockdown enhanced and overexpression attenuated inflammatory genes in $CD14^{+}$ monocytes. *DRAIR* interacted with chromatin at multiple sites and with the methyltransferase G9a to mediate gene regulation. Mechanistically, DRAIR could inhibit promoter G9a recruitment and decrease repressive H3K9me2 levels to enhance target anti-inflammatory gene expression. G9a was upregulated in T2D and its knockdown increased anti-inflammatory genes further supporting its function in DRAIR actions. Mouse *Drair* was also downregulated in peritoneal macrophages from T2D db/db mice. *Driar* knockdown upregulated inflammatory genes in mouse RAW macrophages *in vitro*, and in thioglycolate elicited peritoneal macrophages from C57BL/6 mice *in vivo* demonstrating that its anti-inflammatory functions are conserved across species.

Conclusion. Collectively, our results show that *DRAIR* regulates anti-inflammatory phenotype of monocytes/macrophages via epigenetic mechanisms, and its downregulation in T2D contributes to chronic inflammation. Augmentation of lncRNAs like *DRAIR* could serve as novel anti-inflammatory therapies for diabetic complications.

P15. DIVERGENT TRANSCRIPTION OF THE NKX2-5 LOCUS GENERATES TWO ENHANCER RNAS WITH OPPOSING FUNCTION

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Background. Epigenetic control of gene expression is a critical factor during cardiac hypertrophy, failure and aging. Long non-coding RNAs (lncRNAs) are a class of molecules that govern a large range of functions and play an important role during organogenesis and in disease onset. Since they have specific spatio-temporal expression, their characterization could allow for a better understanding of cardiovascular development and of the epigenetic modifications that occur during heart failure. Thus, they are promising targets for new therapeutic strategies.

Methods. The criteria of selection has been the proximity to genes enriched in cardiomyocytes, the existence of human orthologues, and, furthermore, possessing an overlap with the enhancer regulatory regions. Using a bioinformatics approach, we identified two ncRNAs, we named them *IRENE-SS* and *-div*, upstream of *Nkx2-5*. We functionally characterized the two transcripts through a GapmeR silencing approach in primary culture of mouse neonatal cardiomyocytes.

Results. The two eRNA showed an opposite function in the regulation of nearby gene *Nkx2-5*. RNA sequencing was conducted to determine gene expression modification induced by silencing: we observed an enrichment of pathways modulated in an opposite direction by the selectively silencing of the two eRNAs. Based on the fact that they overlap with an enhancer, we evaluated the effect of their silencing on the epigenetic status of this regulatory element in cultured mouse primary neonatal cardiomyocytes. We obtained a result concordant to what observed in transcription. We also investigated the role of transcripts in chromatin architecture through the String&Binders polymer model that recapitulates the experimentally contacts obtained from Hi-C data using an *in-silico* simulation. We simulated the loss of transcripts as a loss of binding sites correlated to H3K27ac on the enhancer genomic region demonstrating a change in locus conformation.

Conclusion. Our results indicated an opposite role of the two ncRNAs in the regulation of nearby gene though the control of the enhancer acetylation. *IRENE-SS* acted as promoter of transcription, instead *IRENE-div* repressed the enhancer activity through the recruitment of the histone deacetylase SIRT1. We identified an RNA based regulatory mechanism controlling the cardiac transcription factor NKX2-5.

P16. CHROMATIN-ASSOCIATED LONG NON-CODING RNAS REGULATE ENDOTHELIAL FUNCTION IN HEALTH AND DISEASE

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Lining the critical interface between circulating blood and vascular wall, endothelial cells (ECs) play vital functions in health and disease. The optimal gene expression in ECs is essential to maintain endothelial homeostasis, and its dysregulation can lead to EC dysfunction, a common mechanism underlying many metabolic and cardiovascular diseases, e.g. diabetes and diabetes-associated vasculopathy. In our study, we aim to identify the role of long non-coding RNAs (lncRNAs) in the functional regulation of ECs. Initially, in ECs subjected to distinct flow patterns promoting or disrupting endothelial homeostasis, we profiled EC transcriptome using RNA-sequencing, in conjunction with chromatin immunoprecipitation (ChIP) and chromatin conformation capture assays (e.g. 4C and Hi-C). We identified an enhancer-associated lncRNA that enhances endothelial nitric oxide synthase (eNOS) expression, aka LEENE. LEENE is co-regulated with eNOS and its enhancer resides in proximity to *eNOS* promoter in ECs. Using various gain- and loss-of-function approach, including CRISPR editing and RNA inference, we demonstrate that LEENE regulates eNOS expression. Mechanistically, LEENE facilitates the recruitment of RNA Pol II to the *eNOS* promoter to enhance eNOS nascent RNA transcription. Knockout of LEENE homologue in mouse resulted in impaired microvascular function, evident in a hindlimb ischemia model. Furthermore, to elucidate the role of these chromatin-associated, enhancer-derived lncRNAs in EC function in a genome-wide scale, we leveraged a novel in situ mapping of RNA-genome interaction technology (iMARGI). Specifically, we characterized the high glucose-induced global DNA-DNA (using HiC) and RNA-DNA (using iMARGI) contact changes in ECs, in parallel to transcriptome change at single cell level (using single cell RNA-seq). We observed a remarkable increase in inter-chromosomal RNA-DNA interactions particularly enriched in super enhancers in ECs undergoing high glucose challenge. Perturbation of such interactions in ECs, exemplified by suppressing a super enhancer-derived LINC00607 RNA, leads to attenuated pro-inflammatory and

pro-fibrotic gene expression encoded by interacting genomic loci and suppressed monocyte adhesion to ECs. Finally, the co-expression gene network identified from RNA-DNA interactome were validated by comparison between diabetic and healthy donor-derived ECs at single cell transcriptome levels. Taken together, our study suggests an emerging important mechanism by which chromatin-associated, enhancer-derived lncRNAs modulate EC gene expression in physiology and pathological conditions.

P17. UNDERSTANDING THE ROLE OF SNHG17 IN MYC-DRIVEN CELL PROLIFERATION

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Background. Our previous CRISPRi study had placed SNHG17 among the top lncRNA candidates required for MYC-driven proliferation of human lymphoid cells. Several publications have linked this lncRNA to cell proliferation and to tumor progression in several cancer types, including lung, colon and breast cancer. However, the role of SNHG17 in lymphoma and its relation to MYC remain unknown. The functional analysis of lncRNAs is complicated by the fact that customary methods to achieve loss of function such as siRNA, shRNA or standard CRISPR-Cas9 do not work reliably. In order to solve this problem, we applied a kind of saturation CRISPR-Cas9 strategy. We used a library of sgRNAs directed to SNG17 to identify acritical spots in the gene sequence where introduction of mutations or indels leads to loss of function.

Methods. We generated a library of 1,115 sgRNAs covering the length of SNHG17. We transduced Cas9-expressing Ramos cells, a cell line generated from a Burkitt lymphoma, with this library and cultured the cells for 14 days. We then measured the differential depletion of guides using next generation sequencing. To validate these results, we performed CRISPR growth competition assays with individual GFP-expressing sgRNA constructs.

Results. We have mapped more than 50 sites along the SNHG17 gene where CRISPR-Cas9-introduced changes lead to loss of function and reduce the ability of the cells to proliferate. We further generated GFP-expressing versions of the 30 most effective sgRNAs and validated 14 of the sensitive SNHG17 sites in CRISPR growth competition assays with flow cytometry.

Conclusion. There are distinct sensitive sites along the sequence of the SNHG17 gene where CRISPR-Cas9 mediated modifications lead to loss of function. We plan to use these “sweet spots” in SNHG17 to focus future work on the functional role of SNHG17 in MYC-driven cell proliferation.

P18. EPIGENETIC UPREGULATION OF LNCRNA ESCCAL-1 HIJACKS ONCOGENIC PATHWAYS IN ESOPHAGEAL CANCER

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Background. The human genome contains many thousands of long noncoding RNAs (lncRNAs), which are endogenous RNA transcripts with more than 200 nucleotides. LncRNAs lack protein coding potential and are generally expressed at a lower level than protein-coding genes. LncRNAs display more tissue-specific and cell-specific expression patterns. A growing body of evidence has demonstrated that lncRNAs participate in numerous cellular processes and dysregulation of lncRNAs play important roles in tumorigenesis, progression and drug resistance. The transcriptional regulation of aberrant lncRNA expression facilitates the understanding of lncRNA functionality in cancer biology.

Methods. A lncRNA array was used to identify differentially expressed lncRNAs between esophageal squamous cell carcinoma (ESCC) and adjacent normal tissues. qPCR assays were used to confirm the lncRNA, ESCCAL-1 expression in independent patient cohorts (n=100) and patient-derived cancer cell lines (n=5). Whole genome bisulfite sequence (WGBS) was carried out on 10 paired ESCC and normal tissue counterparts for DNA methylation profiling. Short hairpin or siRNA techniques were utilized for lncRNA ESCCAL-1 knockdown in cancer cell lines and functional impact of ESCCAL-1 silencing was examined *in vitro* using proliferation, migration and invasion assays and *in vivo* using mouse xenograft experiments. Chromatin Immunoprecipitation (ChIP) followed by PCR detection was performed to assess the binding of the transcription factor YY1. RNA-seq was performed to identify the gene regulatory network regulated by ESCCAL-1. The esophageal cancer cohort from The Cancer Genome Atlas (TCGA) was used for orthogonal validation studies.

Results. The lncRNA transcript ESCCAL-1 (alias for CASC9) showed increased expression in ESCC relative to adjacent normal tissue by lncRNA array and RNA-seq

data. Higher expression of ESCCAL-1 was verified in independent TCGA patient cohorts and different types of cancers. ESCC patients with higher expression of ESCCAL-1 exhibit worse overall survival. Mechanistically, our whole genome bisulfite sequence revealed that loss of DNA methylation at the promoter region of ESCCAL-1 increases promoter accessibility of the transcription factor YY1 and resulted in transcriptional activation and higher expression in tumors. Knockdown of ESCCAL-1 using siRNAs reduced cellular growth, migration and invasiveness of patient-derived ESCC cells *in vitro* and decreased tumor size *in vivo* in tumor xenografts, suggesting a cancer promoting function. “Guilty-by-association” co-expression analysis of RNA-seq data revealed ESCCAL-1 related gene expression modules were enriched in cell cycle pathways, RNA binding and the Myc pathway. Furthermore, overexpression of ESCCAL-1 activated the PI3K-Akt pathway.

Conclusion. LncRNA dysregulation is an emerging but poorly understood feature of oncogenesis. We reported ESCCAL-1 overexpression in ESCC, which is also overexpressed in other cancer types. Overexpression of ESCCAL-1 promotes cancer cell growth, invasion and metastasis. We discovered that loss of methylation in its promoter and an increase of YY1 transcription factor binding is a principle molecular mechanism of ESCCAL-1 dysregulation in ESCC, resulting in cell cycle and ribosomal pathway dysfunction and PI3K-Akt pathway activation. Epigenetically reprogramming ESCCAL-1 expression using CRISPR/Cas9 technology is under investigation.

P19. THE T1D-ASSOCIATED LNCRNA LNCBACH2 MODULATES PANCREATIC B CELL APOPTOSIS VIA BACH2 REGULATION

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Background. Type 1 diabetes (T1D) is a complex autoimmune disease in which genetic factors interact with environmental factors (e.g. viral infections or dietary components) to trigger an autoimmune attack against insulin-producing pancreatic β-cells. The majority of T1D-associated single nucleotide polymorphisms (SNPs) are located in non-coding regions of the human genome. Many of these SNPs have been predicted to affect the expression, secondary structure and function of long non-coding RNAs (lncRNAs). However, the exact mechanisms by which most of these lncRNAs contribute to the pathogenesis of T1D remain unknown.*LncBACH2* is a lncRNA harboring a SNP associated with T1D (rs3757247) which co-localizes in the human genome with a T1D-associated coding gene named *BACH2*. Interestingly, *BACH2* coding gene has been implicated in cytokine-induced pancreatic β-cell apoptosis.

Against this background, the aim of this work is to characterize the potential function of *lncBACH2* in pancreatic β-cell dysfunction.

Methods. All functional in vitro studies were performed in the human pancreatic beta cell line EndoC-βH1. Overexpression and knockdown experiments were performed by transfection of overexpression vectors or specific siRNAs. Cellular localization of *lncBACH2* was assessed by analyzing its expression in nuclear and whole cellular lysates.

Results and conclusions. The expression level of *lncBACH2* was increased in pancreatic β-cells after 48h stimulation with pro-inflammatory cytokines (e.g. IL-1β + IFNγ). While *lncBACH2* was preferentially located in the cytoplasm of pancreatic β-cells in basal condition, after a pro-inflammatory stimulus, *lncBACH2* was translocated to the nuclei. Inhibition of *lncBACH2* exacerbated cytokine-induced

BACH2 gene expression at the mRNA level, but induced a significant decrease in BACH2 protein expression. Previous studies have demonstrated that *BACH2* inhibition increased cytokine-induced beta cell apoptosis. In line with these data, we observed that *lncBACH2* silencing increased the caspase 3/7 activity, suggesting a potential role of lncBACH2 in the regulation of inflammation-induced apoptotic pathways in β-cells. Further studies are needed to clarify whether the impact of lncBACH2 in pancreatic beta cell apoptosis is through BACH2 regulation.

In conclusion, our results show that *lncBACH2* participates in pro-inflammatory cytokine-induced pancreatic β-cell apoptosis, most probably through regulation of the anti-apoptotic T1D candidate gene *BACH2*. The exact molecular mechanisms by which lncBACH2 participate in beta cell apoptosis require further functional studies.

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P20. CARMN IS AN EVOLUTIONARILY CONSERVED SMOOTH MUSCLE CELL-SPECIFIC LNCRNA THAT MAINTAINS CONTRACTILE PHENOTYPE BY BINDING MYOCARDIN

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Rationale. Vascular homeostasis is maintained by the differentiation of vascular smooth muscle cells (VSMCs) into a contractile phenotype expressing a unique repertoire of functional genes. The landscape of protein coding genes comprising the transcriptome of differentiated VSMCs has been well defined but many gaps remain including an emerging roles of non-coding genes.
Objective. To identify and characterize the function of an evolutionarily conserved, novel SMC-specific lncRNA.
Methods and Results. Using a de novo analysis of a large-scale, publicly available RNA-seq and single cell RNA-seq datasets from multiple tissues and cell types, we have unambiguously identified CARMN (CARdiac Mesoderm Enhancer-associated Non-coding RNA) as a highly abundant and conserved SMC-specific lncRNA. CARMN was initially annotated as the host gene of the MIR143/145 cluster and recently reported to play a role in cardiac differentiation. However, the cell types expressing CARMN in vivo, necessary for a more complete understanding of its function, remain undefined. To this end, we generated a novel Carmn GFP knock-in reporter mouse model and found that Carmn expression is highly restricted to SMCs in vivo. We also found that Carmn is transcribed independently of Mir143/145, is expressed only transiently in embryonic cardiomyocytes and thereafter becomes restricted to SMCs in both adult humans and mice. CARMN expression is dramatically decreased by vascular disease in humans and murine models. Loss- or gain-of-function studies show that CARMN regulates the contractile phenotype of VSMCs in vitro. In vivo, the over-expression of Carmn markedly attenuated injury-induced neointima formation and smooth muscle dedifferentiation in a rat carotid artery balloon injury model. Mechanistically, we found that Carmn physically binds to the key

transcriptional cofactor myocardin, facilitating its activity and thereby maintaining smooth muscle contractile phenotype.
Conclusions. CARMN is an evolutionarily conserved SMC-specific lncRNA that plays a previously unappreciated role in maintaining VSMC contractile phenotype and is the first non-coding RNA discovered to interact with the myocardin/SRF complex.

P21. THE LONG NONCODING TRANSCRIPT LASI MODULATES AIRWAY EPITHELIAL CELL RESPONSES TO TOBACCO SMOKE AND ASSOCIATED CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background. COPD or Chronic Obstructive Pulmonary Disease is the third leading cause of death globally and is projected to increase in prevalence with the world's aging population. Airway epithelial cells (AECs) play a pivotal role in COPD pathology. Chronic exposure to tobacco smoke (TS) alters AEC architecture and secretory goblet cell hyperplasia (GCH) is observed along with innate immune activation, and higher cytokine and chemokine secretion. Till date, therapies for treating COPD are mostly palliative and targeting canonical inflammatory pathways has had limited success. It is noteworthy that TS exposure induces epigenetic changes and alters the expression of long noncoding RNAs (lncRNAs). Therefore, we examined the role of our recently identified long noncoding RNA antisense to ICAM1 or LASI in TS responses of AECs and assessed its clinical relevance in COPD patient samples.

Methods. Bronchial biopsies from active and former smokers with or without COPD and with well-defined disease severity were screened for lung tissue LASI expression and associated inflammation. RNA fluorescent in-situ hybridization (RNA FISH) was employed to evaluate airway epithelial-specific LASI expression. Single molecule resolution and highest sensitivity assay was developed for subcellular localization and co-localization with potential downstream effector molecules. Inflammatory responses and mucus expression levels were evaluated. Results were corroborated in-vitro using primary human bronchial epithelial cells (HBECs) from COPD patients and healthy controls grown in three-dimensional air-liquid interface (ALI) cultures and were treated with TS and/or interleukin (IL)-13 to mimic chronic infection. The role of LASI was interrogated by blocking its expression using small-interfering RNAs

(siRNAs). Molecular and high-resolution microscopy analyses were performed. LncRNA levels of LASI were measured and the TS-induced inflammation and mucus secretory responses were evaluated. Expression levels of IL-6, IL-8, intercellular adhesion molecule (ICAM)-1 and secretory mucin MUC5AC with its transcription factor SPDEF were evaluated.

Results. Ex-vivo analysis of lung tissue biopsies showed a strong association between COPD severity and LASI expression with more than four-fold increase in LASI levels in advanced stage COPD patients. We further found a direct correlation between LASI expression and mucosal immune responses in both lung tissue homogenates and in airway epithelium by in-situ analysis. The findings were corroborated using in-vitro ALI HBEC cultures and observed that LASI preferentially localizes in nuclear/perinuclear region and showed maximum expression in mucus secretory cells. A correlation between LASI expression and increased inflammatory response was observed with a rapid increase in IL-6 and IL-8 levels following TS challenge. There was GCH, showing increased ICAM-1 and MUC5AC expression. A two-hit TS and IL-13 treatment exacerbated LASI expression along with higher inflammatory responses. Most importantly, siRNA-mediated blocking of LASI expression was able to significantly reduce AEC inflammation and mucin expression.

Conclusions. Taken together, these data suggest that LASI expression correlates with COPD severity and blocking its expression suppresses airway mucous and inflammatory responses, suggesting that LASI acts as a crucial mediator of airway epithelial immunity. Thus, LASI may serve as a potential biomarker for lung tissue remodeling and COPD progression and could also be targeted for alleviating the COPD associated pathologies.

P22. LONG NONCODING RNAS OF UPPER RESPIRATORY MUCOSA ARE ASSOCIATED WITH SARS-COV-2 INDUCED MUCOINFLAMMATORY RESPONSE

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Background. Recent clinical studies as well as the *in-silico* retrospective analyses revealed that noncoding transcripts including long noncoding RNAs (lncRNAs) play a critical role in host-pathogen interactions during viral infections such as coronavirus disease 2019 (COVID-19). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the COVID-19 causative agent, gains entry via respiratory mucosa and any dysregulation during this interaction ensues the pulmonary and extrapulmonary complications. SARS-CoV-2 induced inflammatory factors control mucin expression and drive airway tissue remodeling whereby the severe inflammation can cause mucus hyperexpression and could lead to acute respiratory distress syndrome (ARDS). Managing this immune response is among the major challenges faced in achieving the effective treatment. The current study analyzes the respiratory mucosal lncRNAs and mucoinflammatory responses during SARS-CoV-2 infection.

Methods. Nasal swab samples from COVID-19 subjects (n=13, 8M/5F, 71±3.5 y age) were used for RNA isolation and paraformaldehyde-fixed nasal cells were analyzed for the expression of lncRNAs and mucoinflammatory factors. Separately, the 3D airway tissue models of primary human respiratory tract epithelial cells (RTECs) infected with SARS-CoV-2 clinical isolate (USA-WI1/2020, BEI Cat # NR-52384) were also analyzed at 0, 1, 4, 24 and 48 h post infection (hpi). The endpoints analyzed included viral load (SARS-CoV-2 Nucleocapsid), mRNAs for viral entry regulating host factors (ACE-2 and TMPRSS-2), the innate inflammatory factors (IL-6, ICAM-1), respiratory mucins (MUC5AC, MUC5B, MUC2 and MUC4), and the mucin regulatory transcription factors (SPDEF and FOXA3). LncRNAs were analyzed by qRT-PCR and the sub-cellular localization of viral RNAs (vRNAs) and lncRNAs was depicted by

dual-FISH (Fluorescent in-situ hybridization) and immunostaining for epithelial cell marker, pan-cytokeratin (panCK).

Results. In this small cohort of COVID-19 subjects, there was a two log-scale difference in vRNA of subjects with high (n=6) versus low (n=7) viral load. Subjects with high viral showed significantly higher expression of innate inflammatory factors like *IL-6*, *ICAM-1*, and *CCSP* along with the elevated expression of respiratory mucins, MUC4, MUC5AC and MUC5B and the associated transcription regulators, *SPDEF* and *FOXA3,* compared to subjects with low viral load. Interestingly, our recently identified novel lncRNAs, *LASI* (lncRNA on antisense strand to ICAM-1), and *TOSL* (TNFAIP3-opposite strand lncRNA) as well as lncRNA *NEAT-1* were significantly higher in subjects with higher viral load whereas there was no change in ACE2 and TMPRSS2 expression. In our cell culture model studies, SARS-CoV-2 clinical isolate productively infected 3D human RTEC model with highest expression of SARS-CoV-2 N vRNA at 24 hpi, and showed significant increased expression of mucins (MUC2, MUC4, MUC5B, and MUC5AC), inflammatory factors and *LASI*, *TOSL,* and *NEAT1* at 1 hpi. The dual-FISH staining of *LASI* and SARS-CoV-2 N1 vRNA validated that both the transcripts were enriched in nuclear/perinuclear region of RTECs and of panCK+ nasal epithelial cells of COVID-19 subjects.

Conclusions. Together, these data suggest that COVID-19 subjects with higher viral load are impacted by high respiratory mucin expression and the associated inflammatory responses. Interestingly, the lncRNAs, *LASI* and *TOSL* showed associated increased expression suggesting of a possible immunomodulatory role for these lncRNAs in SARS-CoV-2 induced respiratory mucosal responses.

P23. MITOQ REGULATES REDOX-RELATED NON-CODING RNAS TO PRESERVE MITOCHONDRIAL NETWORK INTEGRITY IN HEART FAILURE

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Evidence indicates that mitochondrial network integrity is impaired in cardiomyocytes from failing hearts. While oxidative stress has been implicated in heart failure (HF)-associated mitochondrial network remodeling, the effect of mitochondrial-targeted antioxidants, such as mitoquinone (MitoQ), on the mitochondrial network in a model of HF (e.g. pressure overload) has not been demonstrated. Furthermore, the mechanism of this regulation is not completely understood with an emerging role for post-transcriptional regulation *via* long non-coding RNAs (lncRNAs). We hypothesized that MitoQ preserves mitochondrial fusion proteins (i.e. mitofusin), likely through redox sensitive lncRNAs, leading to improved mitochondrial network integrity in failing hearts. To test this hypothesis, 8-weeks old C57BL/6J mice were subjected to ascending aortic constriction (AAC), which caused substantial left ventricular (LV) chamber remodeling and remarkable contractile dysfunction in one week. Transmission electron microscopy and immunostaining revealed defective inter-mitochondrial and mitochondrial-sarcoplasmic reticulum ultrastructure in AAC mice compared to Sham-operated animals, along with elevated oxidative stress and suppressed mitofusin (i.e., Mfn1 and Mfn2) expression. MitoQ significantly ameliorated LV dysfunction, attenuated Mfn2 downregulation, improved inter-organellar contact, and increased metabolism-related gene expression. Moreover, our data revealed that MitoQ alleviated the dysregulation of a Mfn2-associated lncRNA (i.e. *Plscr4*). In summary, the present study supports a unique mechanism by which MitoQ improves myocardial inter-mitochondrial and mitochondrial-SR ultrastructural remodeling in HF by maintaining Mfn2 expression *via* regulation by a lncRNA.

P24. A LONG NON-CODING RNA, THAT HARBORS A SNP ASSOCIATED WITH BASAL AND GLUCOSE-STIMULATED INSULIN LEVELS, REGULATES THE EXPRESSION OF THE TYPE 2 TRANSGLUTAMINASE GENE IN PANCREATIC ß CELLS.

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Background. Transglutaminase 2 (TGM2) is a multi-functional enzyme which catalyzes transamidation reactions or acts as a G-protein in intracellular signaling. TGM2 is expressed in human pancreatic islets and has been implicated in insulin secretion in rodent pancreatic ß cells. Recently, it has been shown that the expression of a lncRNA (*LOC107987281*) that is transcribed from an intron of the *TGM2* coding gene, correlates with the expression of *TGM2* in several human cell lines and tumoral tissues.

Against this background, the aim of the present study was to characterize the potential role of *LOC107987281* in pancreatic ß cell function and in the development of diabetes.

Methods. A SNP (rs2076380) located in an exon of *LOC107987281* (but intronic for *TGM2*) was genotyped in 376 individuals using a Taqman Genotyping assay. *LOC107987281* and *TGM2* expression were determined in a set of human tissues and in the human pancreatic ß cell line EndoC-ßH1. Cellular localization of *LOC107987281* was assessed by analyzing its expression in the nuclear and whole cell RNA fractions. *LOC107987281* overexpression and knockdown experiments in ß cells were performed by transfection of an overexpression vector or specific siRNAs.

Results and Conclusion. Genotyping of rs2076380 in the FLORINASH cohort revealed that this SNP is associated with basal (0 min) and glucose-stimulated (120 min) insulin levels in women (p=0.005 and 0.037, respectively), as well as with the insulin resistance index (HOMA-IR) (p=0.009) and glucose levels (p=0.007).

LOC107987281 was expressed in several human tissues, having its highest expression levels in lung, placenta, heart, kidney and brain. *LOC107987281* was also expressed in the human pancreatic ß cell line EndoC-ßH1 with expression values similar to those observed in liver and spleen. Interestingly, expression of *LOC107987281* and *TGM2* were correlated across the analyzed human tissues (Spearman's R= 0.87(0.59-0.9); p<0.001).

Regarding the localization of *LOC10798728* in pancreatic ß cells, our results demonstrated that this lncRNA is preferentially nuclear, suggesting a potential role in transcriptional regulation. Indeed, inhibition of *LOC107987281* in EndoC-ßH1 cells using siRNAs led to a significant decrease in *TGM2* mRNA expression. In contrast, overexpression of *LOC107987281* induced a 1.9-fold increase in *TGM2* mRNA expression. Altogether, these results suggest that *LOC107987281* regulates *TGM2* expression in pancreatic ß cells.

In conclusion, our study reveals that *LOC10798728* harbors an exonic SNP that is associated with several parameters related to pancreatic ß cell function and type 2 diabetes (T2D). In addition, our results show that *LOC10798728* regulates *TGM2* expression in pancreatic β cells. Taking into account the role of TGM2 in insulin secretion, it is plausible to think that *LOC10798728* might be implicated in the regulation of insulin production and release. Thus, polymorphisms altering its regulatory capacity might impact the function of pancreatic ß cells (e.g. insulin production) and in consequence, contribute to T2D pathogenesis. Further functional studies are needed to clarify its potential implication in the regulation of insulin release in pancreatic ß cells.

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P25. IDENTIFICATION OF THE LNCRNA CHHEAF-1 AS REGULATOR OF CARDIAC FUNCTION IN HEART FAILURE

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The role of lncRNA in cardiovascular diseases is a topic of intense investigation, due to its involvement in cardiac pathophysiology. We screened myocardial-enriched lncRNAs regulated during cardiac stress and we identified interspersed lncRNAs in a mouse model of pressure overload-induced cardiac hypertrophy through transverse aortic arch constriction (TAC). We named one of these lncRNAs “Cardiac Hypertrophy and Heart Failure-1” (*Chheaf-1*), its expression is upregulated in the first days after TAC, keeps increasing until 2 weeks and diminishes thereafter. Down-regulation of its expression *in vivo* through an anti-sense approach with a “GapmeR” resulted in a decrease of TAC-induced cardiac hypertrophy and heart failure. In cells, we found that *Chheaf-1* regulates the expression of an adjacent gene, *Rtn4*, involved in apoptosis. Interestingly, *Chheaf-1* GapmeR not only decreases *Chheaf-1* expression but also *Rtn4*. Lastly, we identified the human homolog for *Chheaf-1* by means of epigenetic marks on the genomic region adjacent to the human RTN4 gene. In conclusion, we identified a new lncRNA, *Chheaf-1,* with a critical role in heart failure induced by pressure overload, and with a human homolog, describing a new target for heart failure treatment.

P26. LONG NON-CODING VIM-AS1 VARIANT 2 IS UPREGULATED BY TRANSFORMING GROWTH FACTOR B IN LUNG ADENOCARCINOMA

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Transforming growth factor β (TGFβ) signaling can regulate the expression of long non-coding RNAs (lncRNAs). Recently, we conducted a high-throughput screen to identify lncRNA expression upon TGFβ1 stimulation, in which VIM antisense RNA 1 (*VIM-AS1*) was upregulated. Although TGFβ1 has been previously associated with better prognosis of lung cancer patients, by repressing invasiveness and metastasis of their tumors, the role of *VIM-AS1* in such context is yet not fully understood. Based on the database Lnc2Cancer 3.0, the low expression of *VIM-AS1* was associated with worst prognosis in lung adenocarcinoma (LUAD) patients, and upon TGFβ1 stimulation, *VIM-AS1* variant (v.) 2, but not v.1, was statistically upregulated in the human LUAD cell lines A549 and H1299, being detected mostly in the nucleus of these cells. Further, *VIM-AS1* silencing reduced the expression of TGFβ responsive genes, and the knockdown of SMAD2, SMAD3, or their combination abrogated the *VIM-AS1* v.2 expression induced by TGFβ1. Hence, our data suggest that *VIM-AS1* possibly plays a novel role in regulating TGFβ signaling in LUAD.

P27. REGULATION OF TYPE I INTERFERON RESPONSES IN HUMAN PHAGOCYTES BY THE LONG NON-CODING RNA LUCAT1

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Antiviral defense and efficient pathogen clearance are hallmarked by transcription of type 1 interferons and interferon-stimulated genes (ISGs). The magnitude and duration of these responses must be tightly controlled by the immune system as dysregulation can potentially lead to autoimmunity. In the past years, it has become clear that long non-coding RNAs (lncRNAs) play an essential role in the regulation of immune genes. Here, we identified the human lncRNA LUCAT1 as a potent regulator of innate immune responses in human myeloid cells.

LUCAT1 expression is highly dynamic and rapidly induced in human macrophages and dendritic cells after stimulation with innate immune ligands such as lipopolysaccharide (LPS), cytosolic DNA or Sendai Virus infection. The lncRNA is primarily located in nucleus as shown by qPCR on fractionated lysates and single molecular RNA fluorescence in situ hybridization (smFISH). Knockdown or knockout of this lncRNA in human phagocytes using short hairpin RNA (shRNA) and CRISPR/Cas9-based strategies, respectively, revealed that LUCAT1 is a negative regulator of inflammatory and interferon responses. We identified the transcription factor NRF2 as one of the major activators of LUCAT1 expression, which can also be controlled by the immunometabolite itaconate. Knockdown of LUCAT1 in human phagocytes leads to excessive expression of proinflammatory cytokines and type I interferons, defining its role as a negative feedback regulator of immune responses in these cells.

Using Comprehensive identification of RNA-binding proteins by mass spectrometry (ChIRP-MS) as well as RNA immunoprecipitation (RIP), we discovered that LUCAT1 binds several heterogeneous nuclear ribonucleoproteins such as hnRNP A2B1 and hnRNP C as well as the DNA-dependent protein kinase complex (DNA-PK). As both hnRNP A2B1 and hnRNP C play an important role in RNA processing and alternative

splicing, we propose that LUCAT1 interferes with these processes in order to restrain the expression of immune genes. A comprehensive analysis of alternative splicing events in LUCAT1-deficient cells using RNA-seq shows an altered splicing pattern which potentially causes the elevated interferon response in the absence of LUCAT1. Overall, this study highlights the role of LUCAT1 as a nuclear lncRNA and its interaction with RNA-binding proteins to restrain interferon responses in human cells.

P28. LONG NONCODING RNA KHPS1 REGULATES PULMONARY VASCULAR REMODELING VIA A E2F1/MIR-1/SPHK1/S1PR2/HIF1A REGULATORY LOOP

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Background. Pulmonary arterial hypertension (PAH) is a progressive and severe disease characterized by pulmonary vascular remodeling. Sphingosine kinase 1 (Sphk1), the main pulmonary contributor to the sphingosine-1-phosphate (S1P) synthesis, is a modulator of immune and vascular function. We have reported that human pulmonary arterial smooth muscle cell (HPASMC) proliferation, a major contributor to pulmonary vascular remodeling, is regulated by Sphk1/sphingosine-1-phosphate receptor 2 (S1PR2) signaling and mircoRNA-1 (miR-1). Sphk1 is also a modulator of hypoxia-inducible factor 1α (HIF1α) which is involved in cell growth, survival, invasion, and angiogenesis. Recently, a novel role of long noncoding RNA (lncRNA) in PAH has been discovered. LncRNA Khps1 originates from the CpG island and overlaps with a tissue-dependent differentially methylated region (T-DMR) of Sphk1. Khps1 activates Sphk1 expression and counteracts apoptosis induced by the transcriptional factor E2F1. Therefore, we hypothesized that the E2F1/Khps1/miR-1/Sphk1/S1PR2/HIF1α signal transduction pathway regulates pulmonary vascular remodeling in PAH via a regulatory loop which promotes PASMC proliferation.

Methods. PASMCs, isolated from PAH patients, were analyzed for Khps1 and E2F1 expression. Khps1 and E2F1 expression was also assessed in HPASMCs treated with S1P or hypoxia and in cells with deficient expression of S1P signaling mediators. Molecular biology techniques were also used to knockdown or overexpress Khph1 or E2F1 in HPASMCs followed by analysis of S1P signaling mediators and cell proliferation. E2F1 regulation of Khps1 was further analyzed using luciferase constructs. Khps1 regulation of miR-1 expression was also analyzed using luciferase constructs and RNA pull-down. The effect of HIF1α on E2F1 expression was analyzed by either overexpression or knockdown of HIF1α.

Results. When compared to controls, both Khps1 and E2F1 expression was increased in PAH patient PASMCs and in HPASMCs treated with S1P or hypoxia. S1PR2 or SPHK1 knockdown decreased E2F1 and HIF1α expression and cell

proliferation. Khps1 and E2F1 are involved in a regulatory loop as knockdown of Khps1 decreased E2F1 expression as well as other canonical S1P signaling mediators while knockdown of E2F1 decreased Khps1 expression and S1P signaling mediators. Conversely, overexpression of either Khps1 or E2F1 lead to increased S1P pathway activation and proliferation. Furthermore, Khps1 and E2F1 exhibited a reciprocal increase in the others expression. Khps1 regulated S1P signaling via direct binding to miR-1 which lead to decreased miR-1 expression to promote increased cellular levels of S1P signaling mediators (S1PR2, Sphk1, HIF1α and E2F1). HIF1α overexpression lead to increased E2F1 expression while knockdown of HIF1α decreased the hypoxia induced upregulation of E2F1.

Conclusion. A E2F1/lncRNA Khps1/miR-1/Sphk1/S1PR2/HIF1α regularly loop plays an essential role in pulmonary vascular remodeling with PAH. Inhibition of lncRNA Khps1 may become a novel target in PAH.

P29. BECKWITH-WIEDEMANN SYNDROME, BARR BODIES, AND BINDING PROTEINS: THE KCNQ1OT1 LONG NON-CODING RNA REGULATES GENOMIC IMPRINTING IN A SHARP-DEPENDENT MANNER

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Background. Genomic Imprinting refers to an epigenetic mechanism that induces parental-specific gene expression in diploid mammalian cells. Disruptions of imprinting have been linked to several human diseases, including Angelman and Prader-Willi syndromes, among others. The Kcnq1ot1 long non-coding RNA (lncRNA) is responsible for paternal imprinting of a conserved one megabase domain in mouse and human. Importantly, the Kcnq1ot1 imprinting cluster has been linked to an overgrowth disorder called Beckwith-Wiedemann Syndrome, presenting with findings ranging from visceromegaly of intra-abdominal organs, abdominal wall defects, and a predisposition for embryonal malignancies. Despite previous studies investigating the regulation and function of the Kcnq1ot1 lncRNA itself, little is known about how the lncRNA is able to distinguish between target and non-target genes in cis or its mechanism of silencing.

Methods. In order to answer these questions, we used a combination of next generation sequencing technologies, including RAP-DNA, RNA-DNA Split-Pool Recognition of Interactions by Tag Extension (RNA-DNA SPRITE), and a new technique to identify protein-RNA interactions called Covalent Linkage Affinity Purification (CLAP). In addition, we engineered a doxycycline-inducible Kcnq1ot1 mouse embryonic stem cell line to complement our studies.

Results. First using CRISPR-interference and our inducible cell line, we demonstrate that Kcnq1ot1 lncRNA is both necessary and sufficient for silencing imprint target genes. Second, we demonstrate that the lncRNA localizes to its targets on DNA

within a defined topological-associated domain. Third using CLAP-Sequencing, we identify an interaction between the Kcnq1ot1 lncRNA and SMRT/HDAC1 Associated Repressor Protein (SHARP) and demonstrate that Kcnq1ot1 silences imprint targets in a SHARP-dependent manner. Interestingly, SHARP was previously shown to play a critical role in Xist lncRNA-mediated silencing of the X-chromosome during Barr Body formation.

Conclusions. Based on these results, we demonstrate that the Kcnq1ot1 lncRNA scaffolds regulatory factors within a defined 3-dimensional chromatin structure, mirroring the actions of another lncRNA, Xist, during Barr Body formation. Given SHARP's interaction with the Xist and Kcnq1ot1 lncRNAs as case examples, SHARP's additional RNA targets identified from our CLAP dataset (including SHARP's own pre-mRNA), and localization patterns of these interacting RNAs on chromatin using RNA-DNA SPRITE, we speculate that SHARP/RNA interactions may serve more generally to coordinate gene regulation in physiologic and pathophysiologic states.

P30. LONG NON-CODING RNA CAMIRT PLAYS A SENTINEL ROLE IN AGING-RELATED HEART FAILURE VIA INTERACTION WITH PHB2 TO MODULATE MITOPHAGY SIGNALING IN THE HEART

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Background. Mitochondrial dysfunction is an important risk factor for myocardial infarction and heart failure in elderly people. Mitophagy, a physiological process that controls the removal of damaged mitochondria, is compromised in aging or failing hearts. In this study, we examined the physiological role of a cardiac-specific lncRNA *Camirt* that can potentially modulate mitophagy in the heart.

Methods. RNA-seq analyses were conducted to identify cardiac specific lncRNAs in hearts derived from young and aging mice. RNA pull-down and RNA-binding protein immunoprecipitation assay were performed to study the lncRNA-protein interactome in the mouse heart. Real time qPCR was used to examine the expression of lncRNAs in aging mouse and human hearts, and stress-induced chronic failing hearts. *Camirt* conditional (flox) knockout mice were created via CRISPR /Cas9 mediated genome engineering, and subjected to the longitudinal echocardiographic and survival studies after cross-bred with αMHC-Cre mice. Transmission electron microscopy were used to examine the mitochondrial morphology in both *Camirt*-cKO and control hearts. *In vitro* studies were conducted with overexpression and/or knockdown of *Camirt* in cultured neonatal cardiomyocytes or HL-1 cells.

Results. RNA-seq analysis and RT-PCR reveal a lncRNA is highly expressed in both mouse and human hearts, with undetectable levels in other vital organs. Furthermore, the expression of this lncRNA is decreased in aging mouse and human hearts, and failing mouse hearts induced by isoproterenol and doxorubicin. RNA

pull-down and RNA immunoprecipitation (RIP) assays identify prohibitin-2 (Phb2), a known mitophagy receptor, as a binding partner for this lncRNA. Thus, we name this novel lncRNA as a cardiac-specific mitophagy-associated RNA transcript (*Camirt*). Mice with cardiac specific deletion of *Camirt* (*Camirt*-cKO) display progressive heart failure and die within 12 month after birth. RNA sequencing and gene ontology analysis revealed that genes involved in mitophagy signaling were significantly altered in the *Camirt*-cKO hearts compared with the littermate wild type mice. Electron microscopy analyses reveal excessive accumulation of mitolysosomes in cardiomyocytes derived from the *Camirt*-cKO mice. Annexin-V/PI staining showed an increased number of live cells and decreased number of apoptotic cells in NCMs with overexpression of *Camirt* following oxidative stress induced by 2-hour treatment of 1 mM H2O2. Increased autophagy (or mitophagy) activity was observed in HL-1 cells with stable overexpression of *Camirt* and in the presence of chloroquine (an inhibitor for the lysosome degradation). While reduced Camirt expression via shRNA knocking down *Camirt* leads to compromised autophagy (or mitophagy) activity in HL-1 cells. Further biochemical studies support the function of *Camirt*/Phb2 in maintenance of mitochondria function and mitophagy signaling under stress conditions.

Conclusion. Overall, our results suggested that *Camirt* plays a sentinel role in aging-related heart failure via interaction with Phb2 to modulate mitophagy signaling in the heart. Future studies will focus on elucidating the in vivo role and mechanisms of *Camirt* in modulation of mitophagy under natural aging or stress-induced pathologic conditions using the loss- or gain-of-function of *Camirt* mouse models.

P31. A CHROMATIN-REGULATED BIPHASIC CIRCUIT COORDINATES IL-1B MEDIATED INFLAMMATION AND TRAINED IMMUNITY

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Background. Inflammation is characterized by a biphasic cycle consisting initially of a pro-inflammatory phase which is subsequently resolved by anti-inflammatory processes. The coordination of these two disparate states needs to be highly controlled, suggesting that the regulation of the cytokines that drive these processes are intimately linked. Interleukin-1 beta (IL-1β) serves as a master regulator of pro-inflammation has been shown to be a key driver for the establishment of trained immunity. Analysis of the IL-1β genomic locus revealed that *IL-1β* is located within the same topologically associating domain (TAD) as *interleukin-37* (*IL-37*), a powerful anti-inflammatory cytokine, which diametrically opposes the function of *IL-1β*. Guided by the position of a common single nucleotide variant that is associated with inflammatory disease and trained immunity, we identified a novel long non-coding RNA within the *IL-1β* TAD, which we called AMANZI. Interestingly, AMANZI emanated from a region within the promoter of *IL-1β*, which displayed marks of an active enhancer. We hypothesized that AMANZI regulated *IL-1β* transcription and was important for inflammation and the establishment of trained immunity.

Methods. The coordinates of AMANZI were mapped using 3' RACE. AMANZI expression was detected using quantitative real-time PCR. Reduction of AMANZI expression was achieved by RNAseH targeted degradation by introducing antisense oligonucleotides into monocytes and the use of CRISPR mediated deletion of AMANZI in a THP-1 monocyte cell line. Chromatin organization of the region was studied by 3C. ChIP-qPCR was used to interrogate transcription factor binding at regions of interest. The role of AMANZI in trained immunity was examined in human monocytes that were trained with β-glucan *in vitro*.

Results. Transcriptional activation of *IL-1β* resulted in the concomitant expression of AMANZI, which mediated a dynamic long-range chromatin contact with the promoter of *IL-37*. The establishment of this contact led to the activation of *IL-37*

transcription. Importantly, the prerequisite formation of this chromatin contact for *IL-37* expression, inherently introduced a temporal delay which in turn coordinated the biphasic nature of inflammation, with anti-inflammation being temporally delayed from pro-inflammation. Depletion of AMANZI prevented *IL-37* expression through the loss of Mediator and Pol II recruitment to the locus. The loss of the *IL-37* anti-inflammatory response resulted in *IL-1β* over-expression and enhanced trained immunity. In human monocytes, we observed that a naturally occurring polymorphism could modulate inflammatory outcomes by tilting the relative levels of *IL-1β* and AMANZI. Individuals who produced more *IL-1β* showed improved trained immunity and protection against infection, whilst individuals who produced more AMANZI (and *IL-37*) were at greater risk for death due to sepsis.

Conclusion. Our work illuminates a novel biphasic circuit that coordinates the expression of *IL-1β* and *IL-37*, thereby regulating the two functionally opposed states of inflammation from within a single TAD. Single nucleotide variants within AMANZI shift the balance of this circuit, predisposing individuals to being more pro- or anti-inflammatory, and as a result, influence disease outcomes. Furthermore, our findings show that AMANZI and *IL-37* are negative regulators of trained immunity and may be important targets for boosting the efficacy of innate immunity vaccines.

P32. UPREGULATION OF THE LONG NON-CODING RNA XIST AND THE IMBALANCE SEX/RATIO OF PULMONARY ARTERIAL HYPERTENSION

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Background. Recent population-based studies indicate a female predominance in pulmonary arterial hypertension (PAH) of around 2 – 4 over men for all races and ethnicities and across all ages that have been studied to date except for HIV-associated and porto-pulmonary hypertension, which tend to occur more often in men. While female gender has long been established as the major clinical risk factor for PAH, male gender doubles the risk of death in PAH. Even if gender is the strongest disease modifier known, the female predominance in PAH incidence remains largely unexplained. Our recent studies demonstrated that the increased expression and activity of the long-noncoding (lnc)RNA-Xist, essential for X-chromosome (X-Chr) inactivation and dosage compensation of X-linked genes, may explain the imbalance sex/ratio of PAH.

Methods. The studies reported now, using a murine model of plexiform PAH, the intersectin-1s (ITSN) heterozygous ($KO^{ITSN+/-}$) mouse transduced with an ITSN fragment (EH_{ITSN}) possessing endothelial cell proliferative activity, as well as human male and female pulmonary artery endothelial cells (PAECs) and lung specimens, in conjunction with molecular (RNA sequencing, RT-quantitative PCR), cell biology/biochemistry (protein assays, Elk1 transcriptional activation assay, cell cycle analysis), morphological (routine histology complemented by morphometric analyses, immunohistochemistry) and functional approaches (murine echocardiography and hemodynamic measurements) to investigate whether this mouse model of PAH shows sex/gender differences in the lung phenotype, similar to human disease.

Results. Our data demonstrate significant sex-centered differences with regard to EH_{ITSN}-induced alterations in pulmonary artery remodeling, lung hemodynamics and p38/Elk1/c-Fos proliferative signaling, altogether leading to a more severe female

mouse lung PAH phenotype. Moreover, the lncRNA-Xist is upregulated in the lungs of female EH_{ITSN}-$KO^{ITSN+/-}$ mice compared to female wt-mice, leading to sex-specific modulation of the X-linked gene Elk1 and its target, the cell cycle regulatory protein, cyclin A1. These two molecular events are also characteristic to female human PAH lung. Importantly, cyclin A1 expression in the S- and G2/M-phases of the cell cycle of syncronized pulmonary artery endothelial cells (PAECs) of female PAH patients is greater vs. controls, suggesting functional hyper-proliferation.

Conclusion. Thus, the lncRNA Xist upregulation leading to females' PAECs sexual dimorphic behavior may provide a better understanding of the origin of sex bias in PAH. Notably, the EH_{ITSN}-$KO^{ITSN+/-}$ mouse is a unique experimental animal model of PAH that recapitulates most of the sexually dimorphic characteristics of human disease.

P33. CIS-ACTING SUPER ENHANCER LNCRNAS AS DIAGNOSTIC MARKERS OF PROGRESSION TO EARLY STAGE BREAST CANCER

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Background. Increased breast cancer screening over the past four decades has led to a substantial rise in the diagnosis of ductal carcinoma in situ (DCIS). Although DCIS lesions precede invasive ductal carcinoma (IDC), they do not always transform into cancer. The current standard-of-care for DCIS is an aggressive course of therapy to prevent invasive and metastatic disease resulting in over-diagnosis and over-treatment. Thus, there is a critical need to identify functional determinants of progression of DCIS to IDC to allow discrimination between indolent and aggressive disease.

Super-enhancers are regulatory regions of DNA that play critical roles in driving expression of genes that define cell fate decisions and importantly, their normal function can become co-opted during tumorigenesis. Recent studies have found that super-enhancers, in addition to promoting other gene transcription, are themselves transcribed producing super-enhancer associated long noncoding RNAs (SE-lncRNAs). These SE-lncRNAs can interact with their associated enhancer regions in cis and influence activities and expression of neighboring genes. Furthermore, they represent a novel, untapped group of therapeutic targets.

Methods. The MCF10A breast cancer progression series is composed of four cell lines that mimic the progression of normal cells (MCF10A) to atypia (AT1), to DCIS (DCIS), and finally, to IDC (CA1). With an integrative analysis of enhancer loci with global expression of SE-lncRNAs in the progression series, we have identified differentially expressed SE-lncRNAs which can identify mechanisms for DCIS to IDC progression. Furthermore, cross-referencing these SE-lncRNAs with patient samples in the TCGA database, we have unveiled 31 clinically relevant SE-lncRNAs that potentially interact with their enhancer to regulate nearby gene expression. To complement SE-lncRNA expression studies, we conducted an unbiased global analysis of super-enhancers that are acquired or lost in progression. To identify super-enhancer regions at each stage in progression, we profiled H3K27ac using

Chromatin Immunoprecipitation in the MCF10A progression series and ranked super-enhancer regions using the ROSE algorithm.

Results. Here we designate SE-lncRNAs RP11-379F4.4 and RP11-465B22.8 as potential markers of progression through regulation of the expression of their neighboring genes. We seek to validate their potential cis-acting mechanism on their associated genes (RARRES1 and miR200b respectively) and to define their roles in progression. Moreover, we classified 403 super-enhancer regions in MCF10A normal cells, 627 in AT1, 1053 in DCIS, and 320 in CA1 cells. Defining the super-enhancers that are gained and lost at each transition reveals critical pathways that change during progression. 383, 684, and 28 super-enhancers were newly acquired at the AT1, DCIS, and CA1 stages, respectively. Conversely, 173, 120, and 259 super-enhancers were lost in AT1, DCIS, and CA1 respectively. Comparison analysis of acquired regions with super-enhancer regions in 47 ER positive patients, 10 Triple Negative Breast Cancer (TNBC) patients, and 11 TNBC cell lines reveal critically acquired pathways including STAT signaling and NF-kB signaling. In contrast, protein folding and local estrogen production are identified as major pathways lost in progression.

Conclusion. Collectively, these analyses identify differentially expressed SE-lncRNAs and acquired/lost super-enhancers in progression of breast cancer important for promoting DCIS lesions to IDC.

P34. KILN IS A NOVEL LONG NONCODING RNA PROMOTING VASCULAR SMOOTH MUSCLE INFLAMMATION

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There is emerging evidence that pathological vascular remodeling is triggered by the initiation of a rapid and robust pro-inflammatory gene program in vascular smooth muscle cells (VSMCs). However, the mechanism, in particular key long noncoding RNAs (lncRNAs) dictating the activation of VSMC inflammation, remains to be elucidated. Here we report a novel lncRNA called M**K**L1-interactive **I**nflammatory **L**ong **N**oncoding RNA (*KILN*), which is massively induced by inflammatory factors. *KILN* is enriched in human VSMCs of different vascular beds, negatively associated with the VSMC contractile phenotype, and abundantly expressed in abdominal aneurysmal compared with normal aortas. Further studies show that *KILN* is transcriptionally activated by the p65 pathway through a predicted NFkB site within its proximal promoter. siRNA-mediated *KILN* knockdown suppresses, while lentivirus overexpression of *KILN* promotes, the expression of a large number of proinflammatory genes in VSMCs. In vitro RNA pulldown and RNA immunoprecipitation assays reveal that *KILN* physically interacts with MKL1, a key activator of VSMC inflammation through the p65/NFkB pathway. Immuno-RNA FISH further confirm that such physical interaction occurs in the cytoplasmic compartment of VSMCs. Similar to *KILN*, knockdown of MKL1 in VSMCs reduces the expression of a subset of proinflammatory genes. IL1β induces the nuclear localization of both p65 and MKL1, which is blocked upon *KILN* depletion. Knockdown of *KILN* abolishes the physical interaction between p65 and MKL1, as well as the luciferase activity of an NFkB reporter. These data suggests that the activation of *KILN* on the proinflammatory gene program in VSMCs is through MKL1/p65-dependent pathway. Mechanistically, depletion of *KILN* facilitates MKL1 ubiquitin proteasome degradation, which involves its reduced physical interaction with USP10, a deubiquitinating enzyme. Collectively, our data define an important molecular pathway comprising a novel VSMC-enriched lncRNA that interacts with MKL1/USP10 to promote VSMC inflammation. These findings reveal a new potentially druggable target pathway to combat vascular diseases.

P35. HIGH RESOLUTION AND TARGETED MAPPING OF LONG INTRONIC ANTI-SENSE TRANSCRIPTS (LIATS) OF CFTR IDENTIFY AN INTRON 11 LIAT WITH DIRECT IMPLICATIONS FOR TRANSCRIPT-DIRECTED NURD -MEDIATED REPRESSION

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Cystic Fibrosis (CF) is a common human genetic disease directly associated with mutations in the *CFTR* gene. The *CFTR* locus spans over a genomic region of 250kb on chromosome 7 (chr-7q31.2) with the numerous alternatively -spliced transcript isoforms that emerge with discrete epithelial and tissue-specific patterns. Moreover, the *CFTR* locus is decorated with many non-coding RNAs with activities that remain undetermined. However, our recent long read sequencing approaches from *CFTR*-expressing and *CFTR* non-expressing cell types by the Single Molecule Real-Time (SMRT) approach with ISO-Seq resolve the identity of unique intronic transcripts oriented on the opposing strand of the *CFTR*transcript that are inversely correlated with the rare cell-type specific expression of *CFTR*. Such approach can articulate the transcriptome with high levels of confidence and resolution in transcript sequences leading to approaches for re-referencing the transcriptome based on normal developmental or diseased states, as the lab has shown from prior studies and analysis. The abundance of specific LIATs from intron 11 and intron 20 are found enriched in cell types that consistently lack *CFTR* expression and detectable *CFTR* transcripts. Moreover, by employing a CRISPR-Display approach we show that a prominent LIAT from intron 11 can repress *CFTR* transcription. Our approach to conduct the CRISPR-Display using the guide RNA fused to the LIAT-11 transcript results in the overall loss of *CFTR* transcripts in the CaCo-2 and CaLu-3 cell lines. As a result of using this technology to capture the proteins associated with the LIAT-11 we used the CRISPR-Display to recover protein RNA interactions. Furthermore, that transcriptional repression, mediated by LIAT-11, is dependent on recruitment of the CHD4 subunit of the **Nu**cleosome **R**emodeling **D**eacetylase (NuRD) complex and is experimentally consistent with our prior studies demonstrating the discrete susceptibility of CFTR gene transcription to HDAC inhibition more recently illustrating a role of CHD4/NuRD in repression studies of

CFTR in vivo. We suggest the experiments proposed in the following will provide new insight as to how LIAT-11 and LIAT-20 impose cell-type specific control on *CFTR* through the NuRD complex and may provide further justification for therapeutic consideration for complementing CFTR expression within context of compromised CFTR transcription by LIAT abundance.

P36. ONCOGENIC LONG NON-CODING RNA TROLL-8 MEDIATES FATTY ACID METABOLISM IN HUMAN BREAST CANCER CELL METASTASIS

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Background. Long non-coding RNAs (lncRNAs) are regulatory RNAs with no or little protein-coding potential. They function as additional regulators of gene transcription either in cis or trans based on their sequence matching or secondary/tertiary structures. They also serve as decoys, scaffolds, or guides to maintain the spatial-temporal architecture of transcriptional and translational programs on either gene expression or cellular events, including cancer metastasis and metabolism.

Methods and Results. TAp63, one member of the p53 family, is a tumor suppressor in breast cancer metastasis and regulates lipid and glucose metabolism. RNA-seq analysis identified its lncRNA targets, which also differentially expressed during breast cancer progression using MCF10 model. Among them, expression of the oncogenic lncRNA TROLL-8 is significantly higher in triple negative breast cancer (TNBC) molecular subtypes and is negatively correlated with TNBC patient overall survival rate. TROLL-8 interacts with proteins that are enriched in metabolic pathways, detected by protein microarray and Ingenuity Pathway Analysis (IPA). Specifically, seahorse assays demonstrated that TROLL-8 increases breast cancer mitochondrial respiration through targeting the glucose, glutamine and fatty acid oxidation pathways. Silencing of TROLL-8 leads to compromised fatty acid oxidation (FAO), which contributes to accumulated long-chain fatty acids (LCFAs) in the breast cancer cells. The rate-limiting enzyme of FAO, CPT1A interacts with TROLL-8 and we hypothesize that CPT1A contributes to TROLL-8 silencing impaired breast cancer migration. TROLL-8 regulates CPT1A activity and acetylation through blocking its physical interaction with the acetyltransferase ACAT1.

Conclusions. Our study emphasized the potential functionalities of the oncogenic lncRNA TROLL-8 in breast cancer metastasis and metabolism through regulating the FAO rate-limiting enzyme CPT1A activity and post-translational modification. Abnormal expression of TROLL-8 can thus be adopted as diagnostic/prognostic biomarkers, or therapeutic targets for breast cancer control and management.

P37. HETEROGENOUS EXPRESSION OF ANRIL REGULATES VASCULAR SMOOTH MUSCLE CELL ADHESION STRENGTH AND PHENOTYPE

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Background. Single nucleotide polymorphisms (SNPs) within the 9p21 locus have the strongest correlation with coronary artery disease (CAD) [1]. However, the 9p21 locus is a non-coding region, making its connection to CAD enigmatic. 9p21 SNPs affect vascular smooth muscle cells (VSMCs) contractility [2], which during CAD switch from a contractile to synthetic phenotype. Using induced pluripotent stem cells (iPSCs) derived from patients homozygous for risk (R/R) or non-risk (N/N) variants along isogenic knock outs (R/R KO), iPSCs are differentiated into VSMCs to study how variants alter phenotype. We hypothesize variable expression of lncRNAs drives heterogeneous gene expression resulting in variable penetrance of a synthetic phenotype.

Methods. RNA Sequencing and hierarchical clustering for integrins was performed on iPSC derived VSMCs from R/R and N/N patients and KO counterparts. Microfluidic channels were designed to produce laminar flow and uniform shear stress confirmed by simulations. VSMCs were seeded in the device, adhered, and then exposed to acute shear stress with the population that detach collected at the outlet (weakly adherent, WA) and the cells that did not detach (strongly adherent, SA) collected after disassociation. Post sorting, WA and SA populations were analyzed.

Results. All differentiated VSMCs stained positive for VSMC markers,, but there were significant differences in size and morphology between R/R and N/N patients as well as within a R/R population, suggesting the presence of synthetic and contractile phenotypes. RNA sequencing revealed R/R VSMCs had reduced collagen binding integrin expression compared to other genotypes. When cells were exposed to uniform shear stress, a greater percentage detached for both R/R patients compared to R/R KO and N/N which correlated with genotypic size differences.

Smaller more circular cells indicative of synthetic phenotype dominated the WA fraction, and there were fewer cells populating the WA fraction for R/R Patient 1 KO line compared to R/R Patient 1; SA cells were more contractile. WA cells also express more *ANRIL*, a lncRNA in 9p21, compared to SA cells, suggesting that it plays a role in regulating VSMC adhesion, promoting the synthetic phenotype, and potentially exacerbating CAD. RNA sequencing of WA and SA cells from a R/R patient also revealed ~3000 differentially expressed genes.

Conclusions. Our microfluidic device was able to sort patient iPSC-derived VSMCs into distinct subpopulations where WA cells exhibited a more synthetic phenotype and SA exhibited a more contractile phenotype. This work provides a new perspective on phenotype heterogeneity regulated by a non-coding locus.

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P38. UNRAVELING THE ROLE OF THE FOXF1 ADJACENT LNCRNA FENDRR IN LUNG DEVELOPMENT AND DISEASE

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Background. Heterozygous point mutations involving the transcription factor gene *FOXF1* on chromosome 16q24.1 or copy-number variant (CNV) deletions of *FOXF1* or its distant lung-specific enhancer located ~ 286 kb upstream and harboring two lung-expressed lncRNAs *LINC01081* and *LINC01082*, have been found in 80-90% of patients with a neonatal lethal lung developmental disorder Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV; MIM: 265380). Interestingly, unlike point mutations, CNV deletions arise *de novo* almost exclusively on maternal chromosome 16. Approximately 1.7 kb upstream to *FOXF1* maps the divergently oriented lncRNA gene *FENDRR (FOXF1* Adjacent Non-coding Developmental Regulatory RNA). Remarkably, severe cardiac defects, e.g. hypoplastic left heart syndrome and single umbilical artery, have been observed in ACDMPV neonates only with CNV deletions encompassing *FOXF1* and *FENDRR.* In mice, *Fendrr* is essential for development of the heart, lungs, and gastrointestinal system; its homozygous loss is embryonic or perinatal lethal (PMID: 23369715, 24381249). *FENDRR* has been shown to function as a competing endogenous RNA, sponging microRNAs and protein factors that control the stability of mRNAs, and as an epigenetic modifier of chromatin structure around gene promoters and other regulatory sites, targeting them with histone methyltransferase complexes. Deregulation of *FENDRR* expression has been causatively linked also to tumorigenesis, resistance to chemotherapy, fibrosis, and inflammatory diseases (PMID: 33513839).

Methods. We have used RNA-seq in lung biopsy or autopsy tissues from ACDMPV patients with heterozygous point mutations involving *FOXF1* or maternal and paternal CNV deletions of the *FOXF1* distant enhancer, leaving *FOXF1* and *FENDRR*

intact. We have also generated transient *FENDRR* and *FOXF1* knockdowns in fetal lung fibroblasts IMR-90 using siRNAs and measured their expression by RT-qPCR and nCounter system (NanoString Technologies).

Results. We have found that the transcript levels of *FENDRR* and *FOXF1* in lung tissue obtained from patients with CNV deletions were reduced approximately by 75% and 50%, respectively, and that their expression was mono-allelic, originating from the non-deleted chromosome 16q24.1. In contrast, ACDMPV patients with *FOXF1* point mutations had biallelic *FENDRR* expression reduced by half. Depletion of *FOXF1* by siRNA in fetal lung fibroblasts resulted in a 50% decrease of *FENDRR* expression, corroborating previous findings in the conditional *Foxf1* knockout mice. Conversely, knockdown of *FENDRR* had no significant effect on the expression of *FOXF1*.

Conclusion. Our data indicate that *FENDRR* expression in the lungs is up-regulated both *in cis* by the *FOXF1* distant enhancer and *in trans* by FOXF1. They also imply an involvement of *FENDRR* in *FOXF1*-related disorders, including ACDMPV (supported by NIH R01HL137203).

P39. A LNC BETWEEN DNA DAMAGE, HEMATOPOIESIS, AND THE VASCULATURE

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Epigenetic regulatory mechanisms involve different molecular processes and are essential to environmental adaptation, including DNA methylation, histone modification, and non-coding RNAs (ncRNAs). Epigenetic changes in response to genotoxic stressors, such as radiotherapy, accelerate the development of vascular events like atherosclerosis and thrombosis. Indeed, cancer survivors have up to an 80% risk of developing cardiovascular events related to therapy. The epigenetic mechanisms by which genotoxic stress trigger the long-term development of vascular disease are not entirely understood. We showed that increased genotoxic stress led to the induction of a specific long-non-coding RNA (lncRNA) from the DLK1-DIO3 cluster, the lncRNA MEG9. We observed that MEG9 loss-of-function decreased cell proliferation and correlated with an increase in caspase-3-dependent cell death.

Furthermore, MEG9 inhibition diminished sprouting angiogenesis while increasing vascular permeability in vitro. To further understand the functional role of MEG9 in the vascular endothelium, we performed an endothelial-specific gene array, followed by gene ontology analysis. These results suggested that the most affected genes after MEG9 loss-of-function were involved in blood coagulation and thrombosis. Indeed, we confirmed that MEG9 inhibition in HUVECs promotes fibrin formation in human plasma. Currently, we are characterizing the role of MEG9 in vivo by using a CRISPR-CAS9 mouse model. Our preliminary data indicate that MEG9 loss-of-function in vivo affects endothelial cells, circulating platelets, and potentially megakaryocyte differentiation. In summary, our data suggest that the lncRNA MEG9 presents a potential protective role in the vasculature in response to DNA damage. We propose further studies that will elucidate the relative contributions of MEG9 in the endothelial compartment and angiogenesis vs. the bone marrow compartment and megakaryopoiesis.

P40. LNCRNA CONTROLS CARDIAC FUNCTION BY REGULATING ALTERNATIVE SPLICING EVENTS IN THE HEART

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Background. Mutations in the human *triadin* gene (*TRDN*) cause cardiac sudden death and catecholaminergic polymorphic ventricular tachycardia. Up- or down-regulated triadin impairs depolarization-induced calcium release. However, mechanisms controlling triadin levels in the heart remains to be elucidated.

Methods and Results. We define a mechanism by which a cardiac specific long non-coding RNA (lncRNA), *LncRAS-1* controls the level of cardiac triadin isoforms. Knockout (KO) of *LncRAS-1* impairs Ca^{2+} transients in cardiomyocytes (CMs). *LncRAS-1* null mice are susceptible to premature death and cardiac arrhythmia in response to isoproterenol challenge, which was also observed in *Trdn* KO mice. In *LncRAS-1* KO CMs, cardiac triadin isoforms were significantly down-regulated. *LncRAS-1* colocalized and interacted with serine/arginine splicing factors (SRSF) SRSF1 and SRp38 in CM nuclei. Finally, *LncRAS-1* is essential for efficient recruitment of SRSFs to *Trdn* mRNA.

Conclusion. These data indicate that *LncRAS-1* regulates levels of cardiac triadin isoforms, at least in part through alternative splicing. This study provides mechanistic detail into how an individual lncRNA can control cardiac function via alternative splicing.

P41. LNCRNA MIR503HG REGULATES ISCHEMIC MUSCLE REVASCULARIZATION IN PERIPHERAL ARTERY DISEASE

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Atherosclerotic occlusions in lower extremities, usually in legs results in Peripheral Artery Disease (PAD). Severe PAD (critical limb ischemia, CLI) results in extensive tissue loss and limb necrosis resulting in amputation. Currently, no therapies are available to revascularize the ischemic muscle and salvage limb from amputation.

RNA-Seq analysis of muscle biopsies from age and gender-matched controls with CLI-PAD patients identified a cohort of differentially regulated lncRNAs in CLI patients. lncRNAs that are statistically different (q>0.05) between healthy and CLI patients were only taken in the study. This cohort of lncRNAs was compared with RNA-Seq data from human endothelial cells cultured under normal growth conditions or Hypoxia serum starvation (HSS, an in vitro model for PAD) to identify endothelial lncRNAs that can play a role in regulating PAD. This strategy resulting in the identification of lncRNA MIR503HG as a candidate lncRNA for preclinical testing of its ability to induce angiogenesis and revascularize ischemic muscle in Preclinical in vitro and in vivo PAD models. qPCR analysis of MIR503HG in normal and HSS human umbilical vein endothelial cells (HUVECs) showed a significant increase in cytosolic and nuclear fractions in HSS HUVECs compared to normal ECs confirming the RNA-Seq data.

Genetic preclinical PAD models use inbred strains including C57BL/6 and Balb/c mice in a mouse model of hind limb ischemia by femoral artery ligation and resection to determine the translational potential of a therapeutic target. While C57BL/6 mice show excellent recovery to hind limb ischemia (used as a preclinical model for PAD patients with intermittent claudication, IC), Balb/c mice show extensive tissue loss and limb necrosis (used as a preclinical model for patients with CLI). Since MIR503HG was one of the top upregulated lncRNAs in CLI patient's muscle biopsies, we hypothesized that 'MIR503HG inhibits PAD muscle revascularization processes'. To test our hypothesis, we used C57BL/6 mice in

preclinical PAD model. Surprisingly, MIR503HG overexpression by delivering MIR503HG expressing plasmid by electroporation in C57BL/6 mice showed a dramatic induction in perfusion recovery (n=7, by day 14, P>0.05) compared to C57BL/6 that received a control plasmid (quantified by measuring microvascular blood flow by Laser Speckle perfusion imager).

Contradicting our hypothesis, this result indicated that MIR503HG induces perfusion recovery. To confirm this result, we delivered control and MIR503HG expressing plasmids into Balb/c skeletal muscle and examined MIR503HG ability to induce perfusion recovery in the PAD model. We did not observe a significant difference in perfusion recovery between mice that received control and MIR503HG expressing plasmids (n=9). In vitro, silencing MIR503HG in HSS HUVECs significantly decreased endothelial angiogenic potential on growth factor reduced matrigel (n=6, P>0.05). This data indicated that the ability of MIR503HG to induce ischemic muscle revascularization is dependent on the availability of downstream molecular regulators in the ischemic vasculature.

Our ongoing experiments plan to use the bioinformatic approaches to identify MIR503HG targets in the differentially regulated coding gene expressed in normal, IC, and CLI patients (from RNA-Seq data) and identify the molecular mediators that are required for MIR503HG to induce perfusion recovery.

P42. CHARACTERIZATION OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR)- ASSOCIATED LNCRNAS IN THE INNATE IMMUNE RESPONSE IN CYSTIC FIBROSIS

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Background. Cystic fibrosis (CF) is due to mutation of an ion channel encoding the CFTR, yielding highly viscous mucus; (chronic) bacterial respiratory infection is the major cause of CF morbidity and mortality. Recent studies have begun to link CF carrier status to an increased risk for a broad range of conditions across organ systems, including asthma and chronic airway infection. We established a model using the human monocytic cell line, THP-1, cultured with CF patient plasma in order to characterize the lncRNA profiles affecting the function of peripheral immune cells in CF.

Methods. Human THP-1 cells (ATCC-88081201) were cultured in RPMI-1640 plus 10% heat-inactivated fetal calf serum and incubated at 37ºC in a 5% CO_2 incubator. THP-1 cells were treated with a final concentration of 100 nM phorbol 12-myristate-13-acetate for 48 h to prompt differentiation into macrophages. THP-1 and THP-1-derived macrophages were then cultured for 9 h in medium supplemented with plasma from a CF proband, their mother or father, or a healthy control (HC) (median ages 7.9, 39.2, 38.5, and 25 years, respectively; males 66.6%, 0%, 100%, and 100%, respectively). Transcriptomes were profiled with a Clariom D human array (Affymetrix), which targets nearly all RNAs (>57,000) and transcripts (>196,000) annotated in GENCODE (release version 22, GRCh38). Bioinformatics and statistical analyses were performed using established bioinformatics pipelines including Transcriptome Analysis Console (version 4.0.2.1.5, Affymetrix) and Partek Flow (version 6.017).

Results. Transcriptomic profiling identified >4,800 differently expressed genes (DEGs) in THP-1 monocytes cultured with plasma from CF probands or HCs, more than half of which were lncRNAs. In contrast, <200 DEGs were identified from

similar cultures of THP-1 macrophages (Fig. A). THP-1 cells incubated with CF or parent plasma yielded clusters distinct from those incubated with HC plasma for all RNAs (Fig. B) while PCA of the lincRNA (lncRNA subset) data separated cells incubated with CF plasma versus parent plasma. Given that lncRNA DEGs were most distinct for CF patients, we focused on the top-ranked lncRNA that was differently-expressed (Fig. C). lncRNA LINC00969 at the 5' end of the genes encoding the mucins MUC4 and MUC20 was upregulated in monocytes exposed to CF versus. HC plasma (FDR $P=6.54x10^{-7}$, fold change 2.62) (Fig. D). LINC00969 is a good candidate for additional study because it is located close to both LPS-induced histone H3K9ac region (Iglesias, Reilly et al., 2012)and mucin-encoding genes, and is more highly expressed in CF than HCs (Fig. D). Our recent analysis (Sanders, Lawlor et al., 2020) determined that LINC00969 is in linkage disequilibrium with CF; further, MUC4 and MUC20 were among the five loci previously associated with CF lung disease.

Conclusions. This study highlights the utility of lncRNA analyses in defining the innate immune response in CF probands versus their parents and HCs. The lncRNA that we identified here (LINC00969) could be important in host defense mechanisms such as mucociliary clearance and microbial killing in CF. Overall, our work supports expanding the field's previous focus on coding RNAs to globally identifying pertinent lncRNAs in CF.

P43. LONG NON-CODING RNA LINC00276 MAY ENCODE MICROPEPTIDES TO MAINTAIN CELLULAR HOMEOSTASIS IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS

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Purpose. Emerging evidence indicates that some non-coding RNA molecules may harbor short open reading frames (sORFs) that code for functional micropeptides that may play a major role in regulating many pathophysiological processes. However, the functions of these remain largely unexplored given their small size. sORFs can act independently as ligands or signaling molecules by engaging with and modulating larger regulatory proteins to fine-tune complex biological systems. In this study, we attempt to identify potential protein-coding sORFs from LINC00276, a non-coding RNA identified in differentiated ARPE-19 cells.

Materials. Bioinformatic analyses based on evolutionary conservation were used to identify sORFs with the potential to encode a conserved micropeptide. Also, we employed the Coding Potential Calculator version 2 (CPC2) algorithm, a novel discriminative algorithm assessing sequence intrinsic features at the DNA/RNA level, to predict high-quality sORFs. The identified sORFs were then cloned into pEGFP-N1 vector in-frame with C-terminus GFP and 6xHis tag. Expression of these constructs in ARPE-19 cells was analyzed by immunocytochemistry and western blotting after transfection.

Results. In a screen of LINC00276 transcripts we identified an evolutionarily conserved sORF with the potential to encode a highly conserved 19 amino acid (aa) micropeptide located in exon 2, which is common to both LINC00276 transcripts. Using CPC2, we identified another sORF encoding a 74 aa peptide located in exon 4, only present in the second transcript of LINC00276. Expression of these constructs in ARPE-19 cells yielded peptides corresponding to the predicted molecular weight of the fusion peptides, detected by western blot. Both micropeptides, of 19 and 74

aa, showed GFP expression but a distinct GFP expression pattern was observed with the 74 aa micropeptide in ARPE-19 cells.

Conclusion. In this study, we provide evidence for the presence of two putative micropeptides encoded in LINC00276. These may play a role in modulating the expression of genes associated with RPE differentiation. Further studies on the identification and functional characterization of the micropeptides are required to elucidate their biological functions and may provide further insight into the cellular role of LINC00276 in regulating RPE characteristics.

P44. MIR-150 PREVENTS MALADAPTIVE CARDIAC REMODELING MEDIATED BY LONG NONCODING RNA, MIAT AND DIRECTLY REPRESSES PRO-FIBROTIC HOXA4

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Background. MicroRNA-150 (miR-150) plays a protective role in myocardial infarction (MI). Long noncoding RNA (ncRNA), MI-Associated Transcript (MIAT) regulates miR-150 function *in vitro* by direct interaction. Concurrent with downregulation of miR-150, MIAT is upregulated in post-MI hearts, and gain-of-function single nucleotide polymorphisms in MIAT are associated with increased risk of MI in humans. Despite the correlative relationship between MIAT and miR-150, their *in vivo* functional relationship has never been established, and molecular mechanisms by which these two ncRNAs regulate cardioprotection remain elusive.

Methods and Results. Using novel mouse models, we demonstrate that genetic overexpression of MIAT worsens cardiac remodeling, while genetic deletion of MIAT protects hearts against MI. Importantly, miR-150 overexpression prevents the detrimental post-MI effects caused by MIAT. Genome-wide transcriptomic analysis of MIAT null mouse hearts identifies *homeobox a4* (*Hoxa4*) as a novel downstream target of the MIAT/miR-150 axis. Moreover, *Hoxa4* is upregulated in cardiac fibroblasts (CFs) isolated from ischemic myocardium and subjected to simulated ischemia/reperfusion. *HOXA4* is also upregulated in patients with heart failure. Lastly, protective actions of CF miR-150 are partially attributed to the direct and functional repression of pro-fibrotic *Hoxa4*.

Conclusion. Our findings delineate a pivotal functional interaction among MIAT, miR-150 and *Hoxa4* as a novel regulatory mechanism pertinent to MI.

P45. ELUCIDATING GENE REGULATORY ARCHITECTURE BY TRANSCRIPTION FACTOR-DEPENDENT NON-CODING RNAS

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Background. Effective pipelines for winnowing the essential regulatory noncoding (nc)RNAs from the background of unproductive noncoding transcription remains a central challenge. We have pioneered an approach that effectively identifies functional lncRNAs tethered to transcriptional enhancers. We have reported that Transcription Factor (TF)-dependent ncRNA profiling defines a specific class of chromatin-bound ncRNAs more predictive of neighboring gene expression than other genome-scale observables (1). Identification of cell type-specific enhancers, their affiliated non-coding RNAs, and the functional relationship between local lncRNA production and enhancer function in controlling their temporal and cell-type specific activation is crucial for understanding development and disease.

Methods. We have utilized deep ncRNA sequencing, genomic analyses of cell-type specific gen regulatory states, TF localization, enhancer functional assays, and lncRNA functional assays to investigate the relationship between enhancer-based lncRNAs and TF-dependent gene regulatory networks (GRNs).

Results. We have utilized TF-dependent ncRNA profiling in combination with genomic and molecular approaches to elucidate TF-dependent GRNs and provide mechanistic insight into transcriptional architecture. We have identified a functional gene regulatory network driven by TBX5-dependent enhancers and associated ncRNAs for cardiac rhythm control (PMID:29280435); identified a heterotypic TF mechanism between NRL and CRX that controls cell-type specific gene expression in distinct mouse retinal photoreceptors (PMID:31915147); and defined key NFATC2-dependent enhancer loci that mediate enhanced human pancreatic β-cell proliferation (in preparation).

We have recently investigated the genomic interdependence of multiple TF loci implicated in genome-wide association studies and the transcriptional basis of human cardiac disease. ZFPM2 (FOG2), GATA4, and TBX5 are all implicated in

human atrial fibrillation risk. TF-dependent ncRNA profiling defined a novel functional interaction between TBX5 and FOG2 at shared genomic locations that defined a cardiac rhythm gene regulatory network. FOG2, a transcriptional repressor without a DNA binding domain, is recruited to enhancers by GATA4. We found that FOG2-dependent gene expression in the mouse heart overlapped with TBX5-, not GATA4-dependent gene expression, including a module of calcium handling genes required for atrial rhythm homeostasis. TBX5 removal and FOG2 overexpression caused highly correlated effects on ncRNA abundance at ATAC-accessible regions genome-wide. Furthermore, FOG2 overexpression only affected enhancer activity at locations of TBX5 co-binding. The TBX5/FOG2 genomic interaction predicted a genetic interaction in which cardiac rhythm abnormalities caused by *Tbx5*haploinsufficiency were rescued by *FOG2* haploinsufficiency.

Conclusions. TF-dependent ncRNA profiling resolved the genomic mechanism of action of FOG2, a non-DNA-binding TF. This work identified a shared functional interaction between TBX5 and FOG2 on an atrial rhythm GRN despite the fact that these TFs do not physically interact. This functional interaction was defined by coregulation of hundreds of enhancers based on the shared impact of TBX5 and FOG2 on enhancer-based ncRNA expression. This study illuminates the transcriptional interactions between GWAS TF loci for cardiac traits and provides mechanistic clarity to the specific role of individual TFs within the cardiac network. Overall, we illustrate that TF-dependent enhancer transcription can identify functional interactions in TF-dependent gene regulatory networks and unveil TF-dependent genomic architecture that reveals transcriptional mechanisms.

P46. CRISPR MUTATION REVEALS A PHYSIOLOGICAL ROLE FOR MALAT-1 INTERACTION WITH MIR-15/16

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Background. Several studies have documented a possible function for the abundant lncRNA, MALAT-1 (Metastasis Associated Lung Adenocarcinoma Transcript 1), as a competing endogenous RNA (ceRNA) that 'sponges' miRNAs and limits their activity against other target mRNAs. miR-15/16 is a widely and highly expressed tumor suppressor miRNA family important for cell proliferation and survival. We previously showed that this miRNA family also plays important roles in the differentiation of T cells.

Methods. We performed comparative Argonaute-2 high throughput sequencing of crosslinking immunoprecipitation (Ago2 HITS-CLIP, or AHC) and gene expression analyses in normal and miR-15/16 deficient cells. CRISPR targeting with homology directed repair was used to generate mice with a 5 nucleotide mutation in the miR-15/16 binding site in MALAT-1. These mice and mice with conditional targeting of miR-15/16 were subjected to viral infection, tumor challenge, and allergic airway sensitization and challenge (a model of asthma).
Results. MALAT-1 contains the single strongest miR-15/16-dependent AHC signal in T cells, and this signal is entirely dependent on the presence of miR-15/16. Interrupting this lncRNA:miRNA interaction globally derepresses the expression of miR-15/16 target genes, and produces in vitro and in vivo phenotypes that mirror the effects of miR-15/16 deficiency. miR-15/16 is important for controlling the expansion of antigen-specific T cells and the differentiation of memory cells, affecting both viral responses and tumor immunity. It also plays a critical role in T regulatory (Treg) cells that limit allergic airway inflammation.

Conclusion. The abundant lncRNA MALAT-1 binds to miR-15/16 and limits its activity, producing physiologically relevant alterations in gene expression and cell behavior in vivo. These data have implications in asthma and other immune-mediated diseases, as well as in lung adenocarcinoma and other malignancies where MALAT-1 is overexpressed.

P47. THE ANCIENT GENE MYH7B ENCODES A LONG NON-CODING RNA (LNCMYH7B) THAT HAS PROFOUND EFFECTS ON CARDIOMYOCYTE FUNCTION

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Background. There are three sarcomeric myosin heavy chain genes expressed in human hearts: *MYH6* (α-MyHC), *MYH7* β-MyHC), and *MYH7b.* While the crucial roles of the α-MyHC and β-MyHC proteins are well-defined, *MYH7b* is less understood. Myosin heavy chain 7b (*MYH7b*) is annotated as an ancient gene that encodes a myosin II motor. However, a conserved exon-skipping mechanism prevents expression of protein from that locus in the heart; instead a long-noncoding RNA (lncMYH7b) and miRNA (miR-499) are expressed. While the roles of miR-499 in the heart are well-defined, lncMYH7b was thought to be an evolutionary artifact to preserve the expression of miR-499. However, it was observed that variations of *MYH7b* expression always preceded changes in the other myosin heavy chain genes. Specifically, *MYH7b* expression always decreases before β-MyHC does so, indicating a possible regulatory role.

Methods. We systematically examined all possible active molecules originating from the *MYH7b* locus, using, both knockdown and overexpression, to identify the active molecule(s). We then used RNA-seq to determine the global effect of *MYH7b* in the cardiomyocyte as well as looking at the cellular phenotype via sarcomeric staining and beat rate measurements.

Results. We have found that lncMYH7b is the active regulatory molecule originating from the *MYH7b* locus in the heart, independent of miR-499 and any peptide products. Changes in both the molecular and cellular phenotype of the cardiomyocyte occur due to changes in lncMYH7b expression. A knockdown of lncMYH7b caused gene expression changes across the cardiac landscape. Additionally, these cells exhibited a slower beat rate and a pronounced lack of sarcomere structure, likely resulting from decreased expression of adrenergic signaling proteins and the loss of formin homology 2 domain containing 3 protein (FHOD3), respectively. Perhaps most striking, the ratio of β-MyHC to α-MyHC

decreased at both the RNA and protein level. This ratio is known to be critical in cardiac contractility. This decrease is likely due to the loss of expression of TEAD3, a transcription factor known to enhance β-MyHC expression, in these cells.

Conclusions. lncMYH7b is an intriguing example of an ancient gene fulfilling different roles in the cell based on its alternative splicing. Furthermore, a shift in the myosin ratio is a molecular hallmark of heart failure, positioning this pathway as a therapeutic target for clinical intervention.

P48. A LONG NONCODING RNA, LNCMYOD, MODULATES CHROMATIN ACCESSIBILITY TO REGULATE MUSCLE STEM CELL MYOGENIC LINEAGE PROGRESSION

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In eukaryotic chromatin, much of the DNA is buried within nucleosomes, and accessibility of transcription factors (TFs) to their target sequences is sterically hindered by histones and higher-order chromatin structures. The accessibility of chromatin regions determines the transcriptome landscape of the cell, therefore restrict the identity of the cell type. Nevertheless, the detailed mechanism of how TFs access compact chromatin during lineage commitment needs to be further investigated. Exploration of mammalian genomes has revealed a new class of RNA regulator: long noncoding RNAs (lncRNAs), which plays fundamental roles in diverse biological processes. Mechanistic studies of lncRNA functions indicate that they regulate transcription via chromatin modulation. LncRNAs have emerged to regulate muscle development and differentiation during myogenic lineage progression. However, the participation of lncRNAs during myogenic lineage determination remains largely elusive.

Examination of transcriptomic changes during muscle stem cell (satellite cell, SC) activation and differentiation has identified a lncRNA, *LncMyoD*. *LncMyoD* localizes upstream of MyoD, which is the master regulator of myogenic lineage progression. To investigate the role of *LncMyoD*, we conducted loss-of-function experiments with siRNA treatment targeting *LncMyoD* on cultured SCs. The loss of *LncMyoD* did not interfere with SC proliferation but led to a significant defect in SC differentiation. Further mechanistic study showed that *LncMyoD* exclusively binds with MyoD and not other myogenic regulatory factors to promote transactivation of target genes. To further understand whether *LncMyoD* influences chromatin accessibility during SC activation and differentiation, we performed Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) on *LncMyoD* loss-of-function SCs. Examination of TF motif accessibility changes indicated that the loss of *LncMyoD* reduces the permissiveness of the chromatin environment at myogenic E-box

regions. Supporting this notion, MyoD ChIP-seq signal was dramatically reduced after the loss of *LncMyoD*. Meanwhile, *LncMyoD* ChIRP-seq signal showed a remarkable enrichment across MyoD ChIP-seq signal, suggesting the co-existence of MyoD and *LncMyoD* at specific genomic loci. Previous studies showed that ectopic expression of MyoD in non-myogenic cells results in transdifferentiation into myoblast lineage cells. To test whether *LncMyoD* is involved in this process, we generated *LncMyoD*-knockout (KO) 10T1/2 fibroblast cell lines using the CRISPR/Cas9 system and induced MyoD-mediated transdifferentiation. *LncMyoD*-KO clones underwent cell fusion due to the potent effect of MyoD but could not form large myotubes. We subsequently conducted a rescue study by restoring *LncMyoD* expression during the transdifferentiation process and found that the fusion capacity was rescued in *LncMyoD*-KO clones. These results collectively suggest that *LncMyoD* associates with MyoD to promote myogenic lineage determination.

In conclusion, we demonstrated the novel roles of a lncRNA, *LncMyoD*, in regulating myogenic lineage determination and progression. *LncMyoD* binds directly with MyoD to expose compact E-box–containing chromatin and promotes myogenic regulatory factors to access their target regulatory regions. Furthermore, loss of *LncMyoD* leads to defects in SC differentiation and strongly impairs the reprogramming of non-myogenic cells into myogenic cell lineage. Thus, our results provide new insights into how lncRNAs modulate chromatin accessibility to regulate cell lineage determination and progression.

P49. THE ROLE OF LNCRNA LETHE IN THE REGULATION OF INFLAMMATION AND MACROPHAGE POLARIZATION

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Background. Persistence of the M1 macrophage phenotype, and failure to transition to the regenerative or pro-remodeling (M2) macrophage phenotype plays an indispensable role in diabetic wound impairment; however, the mechanism underlying this relationship remains unclear. We have previously shown that lncRNA Lethe is down-regulated in diabetic wounds and overexpression of Lethe enhances diabetic wound healing, indicating a protective role of Lethe in the pathogenesis of diabetic wounds. In this study, we hypothesized that Lethe modulates inflammation and is responsible for the production of pro-inflammatory cytokines and macrophage polarization.

Methods. To test our hypothesis, we treated the mouse macrophage cell line RAW264.7 with LPS (10 pg/ml) and (20 ng/ml) for 24 h to generate M1 macrophages, or IL-4 (20 ng/ml) for 24 h to generate M2 macrophages after overnight serum starvation. Lethe overexpression was achieved by plasmid transfection. Real-time PCR analysis to quantify relative gene expression, using GAPDH as an internal control for mRNA expression.

Results. M1 polarized macrophages exhibited an upregulation of the M1 and pro-inflammatory markers IL-1b, TNFa, iNos, IL-6, and MIP2. Cells exposed to M2 conditions exhibited an upregulation of M2 markers Mrc1, Arg1, and lncRNA Lethe. Overexpression of Lethe in RAW264.7 macrophage cells resulted in an upregulation of Lethe and also decreased expression of the M1 markers IL6 and MIP2.

Discussion. These findings provide the first evidence that Lethe is involved in the regulation of inflammation and limiting the M1 macrophage phenotype. Dysregulation of Lethe in diabetic wounds may explain the abnormal inflammation and persistent M1 macrophage polarization seen in diabetic wounds. Therefore, lncRNA Lethe may represent a potential therapeutic target to counteract the impaired wound healing response in diabetic wounds.

P50. IDENTIFICATION OF PROGNOSTIC AND IMMUNE-RELATED LNCRNA BIOMARKERS IN LUNG SQUAMOUS CELL CARCINOMA

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Background. Increasing evidence has demonstrated that long non-coding RNAs (lncRNAs) play critical roles in mediating anti-tumor immunity and mechanisms of immunosuppression in lung squamous cell carcinoma (LUSC), thus present great potentials as prognostic and immunotherapy biomarkers. However, immune-related lncRNAs and their prognostic values in LUSC remain largely unexplored and there is a wealth of lncRNA expression data accumulated but understudied.

Methods. We developed a computational approach to identify prognostic and immune-related lncRNA biomarkers from tumor RNA-seq data of LUSC patients (n=504) in The Cancer Genome Atlas (TCGA) database, and further validated the findings in Moffitt LUSC cohort (n=108). Five immune signatures were measured from tumor RNA-seq to denote the tumor immune cell infiltration, including CYT (cytolytic activity score), CTL (cytotoxic T lymphocytes scores), TIS (T cell infiltration score), IIS (immune cell infiltration score, and CD8 (CD8 T cell infiltration score). LncRNAs that are predictive of these immune signatures were identified by elastic-net regression. T cell dysfunction is the most important mechanism of tumor immune evasion. To study the potential role of lncRNAs in tumor immune evasion, the interaction test in Cox-PH regression was used to identify lncRNAs associated with T cell dysfunction, such that the effect of tumor T cell infiltration on survival outcome depends on the expression level of these lncRNAs.

Results. For each immune signature, 30-50 out of ~18,000 lncRNAs were identified by elastic net regression as predictive in TCGA LUSC and validated in Moffitt LUSC cohort. To further validate these lncRNAs biomarkers, we selected the ones positively associated with tumor immune infiltration to build panels of immune-related lncRNA signatures. Single-sample gene set enrichment analysis (ssGSEA) was performed to score tumor samples against these panels. The resulted enrichment scores were highly correlated with the true tumor immune infiltration, suggesting that the identified immune-related lncRNAs can serve as reliable surrogate

biomarkers for tumor immune infiltration. In the T cell dysfunction analysis, we identified 34 lncRNAs that influence the function of tumor immune cell infiltration on patients' survival outcomes in TCGA LUSC patients. Out of the 34 lncRNAs, 19 were identified as positively associated with T cell dysfunction, such that higher level expression decreases the prognostic benefits of T cell infiltration; 15 were identified as negatively associated with T cell dysfunction, such that higher level expression increases the prognostic benefits of T cell infiltration. Three lncRNAs, *LINC01515*, *RP11-379F4.6*, and *RP11-417E7.1* were validated in Moffitt LUSC patients.

Conclusion. Our findings demonstrated the value of lncRNAs in predicting and evaluating tumor immune cell infiltration. We also identified lncRNAs that might be responsible for tumor immune evasion, and suggested the potential of these lncRNAs as candidates for noninvasive prognostic biomarkers and immunotherapy targets.

P51. ELUCIDATING THE TRANS-REGULATORY MECHANISMS OF A LONG NON-CODING RNA DURING MYOGENESIS

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Background. Differentiation and formation of skeletal muscle are regulated by the activity of a series of four transcription factors, Myf5, MyoD, Myogenin (MyoG), and MRF4, collectively known as myogenic regulatory factors (MRFs). Previously, we showed that multiple regions within the enhancer elements of MyoD are transcribed into enhancer RNAs (eRNA). These transcripts consist of non-protein coding RNAs associated with gene regulation. In particular, we identified DRReRNA, a long non-coding RNA of about 2kb, transcribed from MyoD distal regulatory region (DRR) 5kb upstream of MyoD on chromosome 7. We have shown that DRReRNA promotes Pol ll and Cohesin loading to MyoG locus on chromosome 1, increasing Myogenin transcription in trans without significantly affecting expression of the MyoD gene in cis. Our functional assays in primary mouse myocytes and in mouse myoblast C2C12 cell line have shown that DRReRNA is recruited to MyoG gene loci and colocalizes with MyoG nascent transcripts. Reduction of DRReRNA impairs chromatin accessibility at MyoG resulting in the reduction of MyoG transcription and hindering muscle cell differentiation. Despite the important role of this long non-coding RNA during muscle cell differentiation, the mechanism by which it identifies its target/s in trans remains to be understood.

Methods. To close this gap, we are evaluating whether chromosomes 1 and 7 come in close proximity allowing DRReRNA-MyoG interaction and enhancing MyoG transcription. If occurring, such inter-chromosomal interaction may be favored during the transition from myoblast to myotubes and strengthened by DRReRNA

expression. To interrogate this potential mechanism, we leverage chromosome conformation capture assays in combination with high-throughput 4C-seq and HiC-seq data from mouse C2C12 cell line.

Results. Our preliminary results suggest that the genomic region encoding for DRReRNA, corresponding to MyoD distal regulatory region on chromosome 7, interacts with MyoG gene locus on chromosome 1. DRReRNA knockdown correlates with reduction of interaction strength suggesting DRReRNA plays a role in mediating the interaction. We are currently examining the extent of the inter-chromosomal interaction through DNA-RNA fluorescent in situ hybridization (FISH) and CRISPR mediated live cell imaging.

Conclusions. Our preliminary results broaden our understanding of non-coding RNAs acting in trans to modulate the expression of target genes causing an increased strength of interaction of specific loci which resemble that of enhancer:promoter looping known to be mediated in cis interactions by eRNAs in other systems.

P52. LONG NON-CODING RNA SIGNATURES FROM EXTRACELLULAR VESCICLES (EVS) CAN DISTINGUISH HEART FAILURE SUBTYPES FROM PATIENT PLASMA SAMPLES AND ACT AS DIAGNOSTIC MARKERS

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Background. Heart Failure (HF) is a leading cause of morbidity and mortality globally, and a significant source of healthcare expenditure in the United States. HF is the clinical manifestation of abnormal ventricular remodelling in response to stressors, and can be classified as HFrEF (HF with reduced ejection fraction) and HFpEF (HF with preserved ejection fraction). Though pathogenesis and therapeutic approaches of the HF subtypes appear to be quite different, certain signaling pathways are likely common. In this regard, decompensated HF for either subtype portends a worse prognosis. Therefore, the identification of novel biomarkers that can discriminate between HF subtypes, and are associated with the trajectory of disease course may refine clinical management and shed light on signaling pathways unique to each subtype. Extracellular vesicles (EVs), nano-sized bilayered structures carrying proteins, nucleic acids, have recently been identified as mediators of inter-cellular communication in cardiovascular diseases. Importantly, the EV-contained microRNAs have been found to have a role as biomarkers for ventricular remodeling. Here we analyze the extracellular transcriptome (mRNA and lncRNA) in plasma of patients with HFpEEF and HFrEF to determine their discriminatory capacity for the HF subtypes and investigate how they change during decongestion from acute HF.

Method. Small and long RNA-sequencing were performed on RNA isolated from EVs of HFrEF and HFpEF plasma samples from a cohort with acute decompensated HF during admission and following decongestion. Comprehensive bioinformatic analysis revealed several extracellular mRNA and lncRNAs differentially expressed between

HFrEF and HFpEF, and examined the interaction network between these candidate mRNAs and lncRNAs.

Results. Small and long RNA sequencing analysis from plasma ex-RNAs revealed several miRNAs, lncRNAs and mRNAs that are different between HF subtypes. While a relatively small number of miRNAs were differentially expressed between HFpEF and HFrEF, plasma mRNAs and lncRNAs more robustly distinguished the HF subtypes. On comparing the EV transcriptome between acutely decompensated HFpEF (8) and HFrEF (6) samples of patients during admission, 650 lncRNAs were differentially expressed with a minimum fold change of + 1.5 and adjusted p-value of <0.05. These were further filtered for transcripts >500 reads, yeilding 49 significantly differentially regulated lncRNAs of which 45 and 4 lncRNAs were observed to upregulated in HFpEF and HFrEF respectively. A similar analysis for mRNA revealed 26 significantly differentially expressed between the HF subtypes with 12mRNAs upregulated in HFpEF and 10 in HFrEF. EV transcriptome analysis between HFpEF patient samples during admission (8) and after decongestion (8) revealed 27 significantly downregulated lncRNAs after decongestion with padj value cut off <0.05. On examining the co-regulation of mRNAs and lncRNAs between HFpEF and HFrEF, many mRNAs and lncRNA were highly coregulated represented by the interaction network.

Conclusion. Unique differentially expressed genes signatures including lncRNAs, mRNAs along with other RNA fragments that could act as characteristic biomarkers to demarcate HFrEF and HFpEF have been identified. While future validation in an independent cohort may provide putative biomarkers to distinguish HF subtypes, close interaction between the DE mRNAs and lncRNAs suggest a role in disease-related signaling pathways.

P53. HUMAN MACROPHAGE LINCRNA, RP11-10J5.1, SUPPRESS MACROPHAGE APOPTOSIS VIA NETRIN 1

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Background. In recent years, long noncoding RNAs (lncRNAs) have been emerging as important regulators of macrophage biology and related metabolic diseases. LncRNAs do not encode proteins, are more than 200 nucleotides in length, lower in expression than mRNAs, and more tissue and cell-type specific. Despite discovery of functional macrophage lncRNAs, very few studies have focused on lncRNAs that are not conserved in mouse and specific to macrophage subtypes. Thus, through RNA-seq of human monocyte-derived macrophages, we identified 2,776 multi-exon long intergenic noncoding RNAs (lincRNAs). Based on abundance and induction during macrophage activation, we prioritized RP11-10J5.1, a human macrophage-specific lincRNA that was highly induced in "M1 like" (LPS/IFNγ- stimulated) macrophage, but suppressed in the "M2 like" (IL-4-stimulated) macrophage. Here, we uncover the role of RP11-10J5.1 in human macrophage survival and its association with unstable human atherosclerosis.

Methods. THP1 were differentiated to macrophages using 100 ng/mL phorbal-12-myristate-13-acetate (PMA), followed by incubation with 100 ng/mL lipopolysaccharide and 20 ng/mL IFNγ. RNA Fluorescent in Situ Hybridization (FISH) was performed to locate RP11-10J5.1 in macrophage. Single stranded antisense oligonucleotide (ASO) was used to knockdown (KD) RP11-10J5.1 in THP1 macrophages, verified with RT-qPCR. After KD, RP11-10J5.1's role in macrophage survival was examined by measuring changes in Annexin V+ by flow cytometry, and cleaved Caspase 3 via western blot. Control and RP11-10J5.1 KD macrophage samples were subjected to RNA-seq to understand the molecular mechanism of RP11-10J5.1. Relation between RP11-10J5.1 and differentially expressed genes were studied with luciferase reporter assay after treatment of HEK293T cells with RP11-

10J5.1 packaged lentivirus. Potential binding partners to the lincRNA were assessed through RNA Immunoprecipitation (RIP) Assay.

Results. RP11-10J5.1, a human macrophage-specific lincRNA, was highly induced in "M1 like" (LPS/IFNγ-stimulated) macrophage, but suppressed in the "M2 like" (IL-4-stimulated) macrophage. RP11-10J5.1 was localized primarily in macrophage nucleus. RNA-seq of control vs. ASO knockdown (KD) of RP11-10J5.1 and subsequent Ingenuity Pathway Analysis (IPA) suggested RP11-10J5.1's role in macrophage survival. RP11-10J5.1 KD induced macrophage apoptosis, as shown by increased protein expression of cleaved Caspase 3 and Annexin V+ macrophages. RNA-seq also revealed Netrin 1 (NTN1) to be significantly decreased in RP11-10J5.1 KD macrophages, validated with RT-qPCR. Lentiviral overexpression (OE) of RP11-10J5.1 also led to increased NTN1 level in the media. NTN1 promoter- luciferase reporter activity in HEK293T cells with RP11-10J5.1 OE showed induction of luciferase activity. RNA immunoprecipitation also showed RP11-10J5.1 to interact with HIF1∝, which is a known inducer of NTN1 transcriptional activity, suggesting that RP11-10J5.1 may bind with HIF1∝ to regulate NTN1 expression. Furthermore, with RP11-10J5.1 KD, there was decreased expression of several known HIF1∝ downstream target genes, such as IGFBP3, TGF∝, and GAPDH. Of clinical relevance, RP11- 10J5.1 expression was highly upregulated in unstable vs. stable human carotid atherosclerotic plaques.

Conclusion. RP11-10J5.1 may facilitate HIF1∝ binding to the NTN1 promoter region, affecting the apoptotic phenotype of human "M1 like" macrophages. RP11-10J5.1 is also associated with unstable human atherosclerotic plaques, and provides insights into the importance of lincRNAs in clinical diseases.

P54. INVESTIGATION OF NOVEL LONG INTERGENIC NON-CODING RNA FUNCTIONS IN OBESITY-INDUCED ADIPOSE TISSUE INFLAMMATION

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Background. Long intergenic non-coding RNAs (lincRNAs) are important emerging regulators of cellular functions. Dysfunctional adipose tissue, characterized by increased inflammation and insulin resistance, plays a central role in the development of type 2 diabetes (T2DM) and atherosclerotic cardiovascular diseases.

Methods. Deep RNA-sequencing of human adipose during low-dose endotoxemia (N=25) identified novel lincRNAs shown to be regulated by inflammation and were validated in an independent human obese cohort, before and after bariatric surgery. Knock down (KD) of lincRNAs was carried out via lentiviral expression of shRNA in human adipose stromal cells (ASCs)-adipocytes. Biotinylated lincRNA pulldown assay was used to identify interacting proteins by Mass Spectrometry (MS). Spatial visualization of the lincRNA was achieved via fluorescent in situ hybridization (FISH).

Results: LincADAIN was identified as a novel adipose lincRNA and is specifically expressed in adipose, but its expression is reduced in both obesity-induced (chronic) and LPS-induced (acute) inflammation (-77% and 53% respectively, $p<0.05$). Human lincADAIN expression negatively correlates to BMI in obese ($p<0.001$, r2-0.3042) but not lean individuals. KD of lincADAIN in ASC-adipocytes, increased protein but not mRNA levels of inflammatory cytokines in adipocytes such as IL-8 and MCP-1. LincADAIN co-localizes with P-bodies in ASC-adipocytes, visualized using FISH and P-body marker, GW182. MS analysis of biotinylated LincADAIN revealed many central P-body proteins as potential interactors. P-bodies are cytoplasmic ribonucleoprotein granules that are important regulators of mRNA translation.

Conclusions. These results suggest that lincADAIN is a novel lincRNA regulating adipose inflammation potentially through adipocyte P-body modulation of RNA

translation or stability. Investigation of lincADAIN may provide novel mechanistic and genomic insights into adipocyte cellular functions and translational regulation in obesity induced adipose tissue inflammation.

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