CARDIO PULMONARY INSTITUTE

CPI Retreat 2026 | Poster Abstracts

Poster Session I: Areas 1-3, Hub 1-3
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AREA 1

A1-1: Identification of telocytes using novel intersectional genetics approaches

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Telocytes represent a recently described population of interstitial cells distinguished by their long, slender cellular extensions and widespread presence across organs. They have been implicated in tissue maintenance and repair, yet their precise in vivo identification remains challenging due to promiscuous marker expression and pronounced organ-specific heterogeneity. Here, we applied dual recombinase-mediated genetic lineage tracing (Dre and Cre recombinases) to compare three intersectional systems based on the co-expression of CD34–Pdgfra, CD34–cKit, and cKit–Pdgfra. This approach allowed us to assess labeling efficiency and specificity, thereby establishing a refined strategy to identify, characterize, and manipulate the telocyte lineage in vivo.

We found that the CD34-Pdgfra system yields the broadest and most robust labeling of tdTomato reporter-positive cells across multiple organs. By contrast, tdTomato expression is sparse in the CD34-cKit system and is overall reduced in the cKit-Pdgfra system, particularly in the heart. Lineage specificity analysis further revealed that tdTomato-positive cells in all three systems show no overlap with smooth muscle cells, immune cells, or pericytes. Notably, both cKit-Pdgfra and CD34-cKit display substantial endothelial co-expression in the heart; moreover, despite sparse overall labeling, the CD34-cKit system also shows evident endothelial co-expression in other tissues. In comparison, endothelial overlap is markedly lower in the CD34-Pdgfra system. Taken together, these findings identify CD34-Pdgfra as the most suitable system among those tested for tracing telocyte-enriched populations and establish a tractable platform for future studies of telocyte morphology, organ-specific heterogeneity, and function in tissue injury and remodeling.

A1-2: Tumorigenic potential of bronchioalveolar stem cells in Kras-driven lung cancer

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Lung adenocarcinoma (LUAD), one of the leading causes of mortality among cancer patients, is frequently associated with mutations in the Kirsten rat sarcoma virus (Kras) gene and originates from different epithelial cell types. Niche-associated, marker co-expressing bronchioalveolar stem cells (BASCs) display a high regenerative capacity and are instrumental for the repair of the distal lung epithelium. Earlier studies proposed BASCs as potential cellular source for LUAD, but since previous animal models lacked BASC-specificity, definitive proof for this claim was missing.

Here, we combined BASC-specific targeting with selective or stochastic activation of oncogenic Kras (KrasG12D) to delineate the tumorigenic potential of BASCs in vivo. We demonstrate that BASCs are susceptible to malignant transformation, give rise to hyperplastic foci consisting of bronchiolar and alveolar epithelial cells, and serve as cancer-initiating cells upon forced KrasG12D expression. However, none of the LUADs was exclusively derived from BASCs following stochastic KrasG12D activation, although some cells within the developing tumors phenotypically switched towards a “BASC-like” state. Histopathological analysis of LUADs in both models revealed differences in topology, growth pattern, cytoarchitectural features, cellular/nuclear pleomorphism and atypia as well as proliferative capacity.

We conclude that BASCs do not serve as predominant cancer-initiating cell type in Kras-induced LUAD but exhibit strong oncogenic potential, as evidenced by pronounced nuclear atypia and pleomorphism, invasive growth behavior, increased proliferation rate and broader histological diversity of BASC-derived LUADs.

A1-3: Lung cancer progression and fibrosis are interconnected through distinct fibrocyte states

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The frequent coexistence of pulmonary fibrosis and lung cancer suggests shared pathogenic mechanisms, yet the cellular drivers linking these conditions remain poorly defined. Here we identify fibrocytes as central mediators of the tumor-fibrosis axis. Using a newly established mouse model of lung cancer-associated fibrosis, we demonstrate that combined disease significantly exacerbates both tumor burden and fibrotic remodeling compared to either condition alone. Single-cell RNA sequencing resolved distinct fibrocyte populations, including pro-inflammatory and pro-tumorigenic subsets, the latter enriched for glycolytic and metabolic pathways. In parallel, multiple fibroblast lineages associated with immune regulation, stromal remodeling, and tumor progression were identified, highlighting pronounced stromal heterogeneity. Lineage mapping suggested dynamic relationships between fibrocyte and fibroblast states, indicating plasticity within the stromal compartment. Functional assays revealed that fibrocytes promote lung cancer cell proliferation and migration while undergoing enhanced activation and differentiation, consistent with bidirectional tumor-stroma crosstalk. Metabolic profiling demonstrated that fibrocytes exhibit elevated basal and maximal respiratory capacity relative to tumor cells and other stromal populations, supporting a role in shaping the metabolic microenvironment. In an ex vivo model, fibrocytes synergized with tumor cells to amplify extracellular matrix deposition, collagen organization, and fibrotic remodeling. Together, these findings establish fibrocytes as key integrators of inflammatory, metabolic, and stromal signals that couple fibrosis to lung cancer progression. Targeting fibrocyte-driven pathways may provide therapeutic opportunities to disrupt the reciprocal reinforcement of fibrosis and malignancy.

A1-4: DNA methylation-dependent metabolic processes in shaping tumor-associated macrophage function in lung cancer

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The role of DNA methylation in shaping the lung tumor microenvironment, particularly in tumor-associated macrophages (TAMs), remains unclear. This study investigates how DNA methylation regulates TAM development, differentiation, and functional polarization in lung cancer. TAMs were isolated from human lung tumor tissues and matched adjacent healthy lung tissues using multispectral flow cytometry (FACS). DNA methylation profiling was performed using the Infinium HumanMethylation850 BeadChip (Illumina). Differentially methylated regions (DMRs) were annotated relative to gene bodies, promoters, CpG islands, and tiling regions, followed by pathway enrichment analysis. Compared to macrophages from healthy tissue, TAMs exhibited widespread DNA hypermethylation. Hypermethylated DMRs were significantly enriched in genes involved in fatty acid (FA) metabolism, whereas hypomethylated regions were associated with glycolytic pathways. Integrated analysis with transcriptomic data from FACS-sorted TAMs revealed that hypermethylation correlated with downregulation of FA metabolism genes, while hypomethylation was linked to upregulation of glycolytic genes. Among top FA metabolism candidates, FABP4 displayed the most pronounced DNA hypermethylation and lowest mRNA expression. mRNA analysis of DNMTs and TETs showed a significant correlation between DNMT1 and FABP4, which is highly upregulated in TAMs. Functionally, TAMs exhibited a monocytic-like phenotype with suppressed FA oxidation and enhanced glycolysis. Modulating FABP4 in monocytic cells, followed by co-culture with lung cancer cells, reduced cancer cell proliferation. These findings reveal a distinct DNA methylation signature in lung cancer TAMs that selectively represses FA metabolism and promotes glycolysis, regulating TAM function and phenotype. This study highlights DNA methylation-dependent metabolic reprogramming in TAMs as a promising therapeutic target in lung cancer.

A1-5: Tumor-derived initiators of cancer-induced cardiomyopathy: insights from zebrafish and circulating mediators

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One of the major complications of cancer cachexia is “cancer-induced cardiomyopathy”, a heart failure-like condition, whose underlying mechanisms remain poorly understood. Systemic local immune activation are thought to be major contributors to cardiac wasting. However, it remains unclear (1) whether immune infiltration initiates cardiac damage, and (2) which tumor-derived mediators trigger early cardiac dysfunction and promote immune cell invasion into the heart. To address these questions, we use the zebrafish model, which offers unique advantages including genetic tractability and in vivo imaging. We repurposed an inducible model of hepatocellular carcinoma and found that liver tumors drive progressive cardiomyopathy in adult zebrafish, characterized by ventricular remodeling and fibrosis, impaired contractility, and pathological cardiomyocyte dedifferentiation by 4 weeks of cancer-induction. Investigation of early events revealed cardiac cell apoptosis accompanied by neutrophil infiltration. Tumor induction during larval stages confirmed neutrophil infiltration associated with ventricular dilation in tumor-bearing larvae. Ongoing neutrophil ablation experiments aim to determine the causal relationship between neutrophil infiltration, cardiac cell death, and fibrosis. In parallel, we are identifying tumor-derived factors that drive early cardiac damage and immune infiltration through plasma proteomics and tissue transcriptomics. Notably, in our larval model we observe tumor-derived extracellular vesicles both in circulation and adherent to the endocardial lining of the heart in tumor-bearing zebrafish, and we are currently investigating the pathogenic role of these structures in both larval and adult models. Our work establishes the zebrafish as a powerful system to dissect the mechanisms of cancer-induced cardiomyopathy and suggest that tumor-driven immune responses and extracellular vesicles may play key roles in initiating cardiac dysfunction.

A1-6: Variant-dependent recognition of SARS-CoV-2 by the TLR2 axis shapes type I interferon responses in human monocytes

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Background: SARS-CoV-2 infection is associated with distinct monocyte states, ranging from early interferon-driven antiviral responses in non-pneumonic disease to pro-inflammatory programmes in pneumonic COVID-19. However, the mechanisms underlying these divergent responses remain unclear.

Results: In primary human monocytes, Omicron variants, in contrast to pre-Omicron variants, induced a prolonged type I interferon (IFN) and inflammatory response that culminated in cell death. This phenotype could be prevented by blocking IFN receptor. Because monocytes lack the canonical viral entry receptors required for productive SARS-CoV-2 infection, these responses occurred independently of productive viral replication. We therefore hypothesised that SARS-CoV-2 engages and dysregulates plasma membrane-associated pattern-recognition receptors. Although both TLR2 and TLR4 have previously been implicated in sensing structural components of viral particles, blocking antibody experiments and validation in knockout cells identified the TLR2 axis as the key pathway mediating recognition of Omicron variants. Moreover, TLR2 internalisation was required for efficient induction of the IFN response. Because TLR2 has been reported to recognise the viral envelope (E) protein, we compared Omicron and pre-Omicron E protein sequences and identified a single T9I substitution unique to Omicron variants. Notably, MERS-CoV also carries isoleucine at position 9, whereas SARS-CoV retains threonine at this site. Consistent with this observation, MERS-CoV triggered a similar interferon and inflammatory response in monocytes, whereas SARS-CoV did not.

Conclusion: Omicron variants uniquely induce a prolonged, TLR2-dependent type I IFN and inflammatory response in human monocytes, likely linked to the E protein T9I substitution, revealing how coronavirus variant-specific sensing can drive protective versus maladaptive innate immune states.

A1-7: A unified single-cell atlas of mouse tissue damage across vascular disease models

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Single-cell RNA-sequencing (scRNA-seq) offers powerful insights into cellular responses across tissues and disease states.

We present an integrated atlas of 390,000 mouse cells across multiple tissues (heart, carotid, lung, and brain) and disease models, including heart disorder, stroke, and lung injury. To harmonize technical and biological variation across datasets, we applied scVI to integrate raw read counts and effectively removed technical batch effects.

Cell type annotation was performed using curated marker genes from the literature. We compared disease-associated gene signatures with gene–disease associations from DisGeNET and the GWAS Catalog, enabling the identification of overlapping and condition-specific genes.

The atlas provides a versatile framework for downstream investigations. It enables exploration of shared and unique transcriptional programs across diseases, identification of disease-specific cellular states, and analysis of conserved patterns across conditions. Importantly, the learned latent representations from this atlas can be applied to spatial transcriptomics data, allowing inference of disease presence. Another application is the inference of dynamic transcription factor (TF) networks from time-series gene expression data, allowing the identification of stage-specific regulators across disease progression.

To ensure broad accessibility, we developed a user-friendly web-based platform, the VDA app, which enables intuitive exploration and analysis without requiring programming expertise. Users can explore the atlas and its rich metadata, perform differential expression analyses, and investigate gene functions across specific cell types and disease conditions.

A1-8: CHIP-Associated TET2 Mutation Reprograms NK Cells to Drive Valvular Interstitial Cell Activation and Calcification via IFN-γ Signaling

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Clonal hematopoiesis driven by TET2 mutations has been linked to cardiovascular disease, but the role of NK cells in CH mutant patients remains unclear. We hypothesized that TET2-mutant NK cells enhance pro-inflammatory signaling and promote fibroblast activation and calcification.

Single-cell RNA sequencing revealed that TET2-mutant NK cells interact with valvular interstial cells through the IFN-γ signaling pathway, whereas wild-type NK cells do not exhibit this interaction. Functional assays showed that siRNA-mediated TET2 knockdown in NK cells significantly increased IFN-γ and TNF-α production, as confirmed by flow cytometry (P<0.05).

Conditioned media from siTET2 NK cells, but not control (siNC), induced higher expression of osteogenic and fibrotic markers (ALP, COL1A1, SPP1, BMP) in cardiac fibroblasts and valvular interstitial cells (VICs) by qPCR (P<0.05). Immunofluorescence at 48 hours confirmed increased collagen I (P=0.036) and α-SMA (P=0.028), indicating enhanced fibroblast and VIC activation.

After 21 days of treatment, both cardiac fibroblasts and valvular interstitial cells (VICs) exhibited significantly increased calcification under siTET2-conditioned media, as shown by Alizarin Red staining (P<0.05).

UK Biobank analysis confirmed that TET2 mutations independently increase non-rheumatic aortic valve disease risk (OR 1.25, 95% CI 1.01–1.54). Pathway enrichment included IFN-γ-driven immune responses (e.g., lymphocyte immunity, chemotaxis, leukocyte migration) and ossification. Additionally, an NK cell-derived IFN signature was positively linked to disease risk (OR 1.05 per SD, 95% CI 1.02–1.09, P<0.001).

In summary, TET2 deficiency in NK cells promotes a pro-inflammatory phenotype characterized by elevated IFN-γ and TNF-α secretion, driving fibroblast activation and calcification. These findings identify a novel mechanism linking clonal hematopoiesis to valvular disease and suggest NK cells as potential therapeutic targets.

A1-9: T Cell Activation and Clonal Expansion in Chronic Heart Failure

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Chronic heart failure (HF) is characterized by adverse ventricular remodeling and persistent inflammation, leading to progressive cardiac dysfunction. To investigate the contribution of immune responses following myocardial infarction (MI), we combined immunophenotyping, single-cell RNA sequencing, and echocardiographic analysis. MI resulted in a significant decline in cardiac function, with ejection fraction and fractional shortening reduced by ~30–40% (p < 0.001), accompanied by decreases in stroke volume and cardiac output (p < 0.01). Structural remodeling was evident by a ~40–50% reduction in anterior wall thickness (LVAWd) and a significant increase in posterior wall thickness and LV mass (p < 0.01).

Treatment with anti-CCL5 improved cardiac function by ~15–20% compared to MI+IgG (p < 0.05) and attenuated adverse remodeling, whereas abatacept showed moderate but less pronounced effects (~10–15% improvement, p < 0.05). Immunological profiling revealed a reduction in T central memory cells and an increase in $CD4^+$ Th17 and $CD8^+$ effector memory T cells, along with elevated CCR5 expression associated with poor prognosis. Clonal expansion and predicted autoreactivity of T cells were observed, supported by inflammatory T cell infiltration in human and murine hearts post-MI.

These findings highlight a role for sustained T cell–mediated inflammation in HF progression and identify the CCL5–CCR5 axis as a potential therapeutic target.

A1-10: GA-binding protein transcription factor subunit alpha (GABPA) sustains endothelial angiogenic function, histone gene expression and RNA-chromatin structures

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GABPA is a widely expressed transcription factor involved in proliferation and metabolic control. While previously linked to endothelial promoter control and retinal angiogenesis, its role in endothelial cell biology remains unclear. Here, we identify GABPA as a critical regulator of endothelial angiogenic function and uncover a non-canonical mechanism linking GABPA to histone gene expression and RNA-chromatin structures. GABPA knockdown (KD) in human umbilical vein endothelial cells (HUVECs) reduced endothelial proliferation, migration, VEGF-A mediated spheroid outgrowth, and tube formation. To define the underlying mechanism, RNA-seq after GABPA KD was performed. Transcriptome analysis revealed broad suppression of E2F/S-phase, G2/M checkpoint, and DNA replication-associated programs, alongside induction of p53/p21-related stress pathways, a strikingly coordinated downregulation of 48 replication-dependent histone genes. Notably, these genes showed minimal overlap with GABPA chromatin occupancy by CUT&RUN, arguing that their regulation is not primarily mediated by direct promoter binding. To explore alternative mechanisms, a complexome profiling, GABPA immunoprecipitation followed by mass spectrometry identified a nuclear complex containing GABPB1 and the RNA helicase DDX41. Given the regulating roles of RNA helicases on genome-associated RNAs, we examined RNA-DNA hybrid-associated signals. GABPA KD significantly reduced the nuclear S9.6 signal, indicating a role in maintaining RNA-DNA hybrid structures. GABPA RIP-seq identified a strong association of GABPA with substantial RNase H resistant histone transcripts and enrichment in dsDNA RIP-seq, consistent with their incorporation into non-canonical RNA-chromatin assemblies. Together, these data support a model in which GABPA, along with GABPB1 and DDX41, sustains endothelial angiogenic function by maintaining histone gene expression and RNA-chromatin structures, potentially including RNA-DNA triplex interactions.

A1-11: A single-cell transfer RNA expression atlas of human bone marrow hematopoiesis

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Transfer RNAs (tRNAs) deliver amino acids to the protein synthesis machinery, functioning as adapter molecules to translate mRNA into protein. The abundance of tRNAs determines elongational speed and affects nascent protein abundance and folding.

We previously reported differential tRNA supplies in sorted hematopoietic stem cells, committed progenitors and T cells, suggesting a role of tRNA abundance in defining cellular identity. Now, we established the first single-cell tRNA expression atlas of human bone marrow hematopoiesis to comprehensively elucidate cell-specific tRNA expression of the entire hematopoietic system.

tRNA-sequencing is challenging due to sequence redundancy, base modifications and rigid secondary structures. We developed a single-cell tRNA-sequencing approach including a computational workflow that efficiently captures full-length tRNAs and mRNAs simultaneously, which we applied to 18000 human bone marrow cells.

Principal Component Analysis based on mRNA expression enabled us to cluster and annotate cell types, reconstruct lineages, and project differentiation trajectories. We integrated these layers of information with single-cell tRNA profiles, uncovering striking tRNA expression differences between cell types. Importantly, we identified lineage-specific continuous and dynamic tRNA expression changes along all differentiation trajectories using pseudotime analyses. This included the most immature HSPC compartment, with tRNAs changing along megakaryocytic-erythroid, granulocyte-macrophage, monocytic-dendritic and lymphoid lineages.

Moreover, by applying UMAP based on tRNA information and subsequent backtracking of cell types, we characterized tRNA expression alone as highly indicative for HSPCs, dendritic cells and erythroid progenitors.

In conclusion, we present a comprehensive atlas of differential tRNA expression at single-cell resolution across hematopoiesis, changing along differentiation paths and informing about cell types.

A1-12: Regulation of the IL-1:NF-κB pathway in LUAD primary cell lines and tissues in relation to the intratumoral glycodelin level

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Lung cancer is one of the most prevalent cancer types with a high mortality rate. Lung cancer can be differentiated into two subtypes, the non-small cell lung cancers (NSCLCs) and the small cell lung cancers. NSCLCs make up around 85% of lung cancer cases and are mainly comprised of lung adenocarcinomas (LUADs).

In 2015 the embryonal protein glycodelin (encoded by the gene PAEP) was identified as a potential biomarker for NSCLCs because it was found to be re-expressed in around 90% of NSCLCs. Furthermore, a small subset of these tumors showed very high levels of glycodelin and patients with such tumors had a lower survival rate.

The proinflammatory cytokine Interleukin-1 (IL-1) is relevant for lung cancer initiation, because IL-1β blockade in patients reduced lunger cancer incidence in the CANTOS trial. As these accidental observations suggested a link between the IL-1:NF-κB pathway and lung tumor development we decided to analyze human LUAD tissue sections as well as primary cell lines with varying glycodelin levels regarding their regulation of the IL 1:NF κB pathway by single cell and bulk analyses.

The results showed a reduction in the expression of components and effectors of the NF-κB pathway in LUAD cell lines expressing high levels of glycodelin at both protein and mRNA, but not on chromatin level. However, in LUAD tissue sections analyzed by smRNA-FISH the effects were not reproduced, instead an upregulation of some NF-κB pathway components and effectors was detected.

The interesting phenotypes of the patient-derived primary cell lines represent an unexpected and unusual perturbation of the IL 1:NF-κB pathway and, thus, may prove helpful in elucidating the mechanisms by which the pathway affects lung tumor incidence. To define a possible interplay between increased glycodelin expression and IL-1:NF-κB suppression, the findings of the cell lines will be validated in further cell lines, and subsequent experiments modulating glycodelin will be performed.

A1-13: Persistence of alveolar fibroblast-derived ADAMTS4+ cells in a preclinical model of delayed pulmonary fibrosis resolution

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Idiopathic pulmonary fibrosis is the most common and aggressive form of interstitial lung disease. Despite extensive research on the pathomechanisms of fibrogenesis, little is known about the mechanisms of fibrosis resolution. Here, lineage tracing of alveolar fibroblasts was carried out during fibrosis development and delayed resolution in aged mice. Histological analyses, single-cell transcriptomics, and ex vivo models including alveolar organoids and precision-cut lung slice cultures were employed. The data reveal that lipofibroblasts contribute to myofibroblast formation during fibrogenesis, with the reverse differentiation trajectory occurring during fibrosis resolution. Importantly, delayed resolution is associated with the persistence of ADAM metallopeptidase with thrombospondin type 1 motif 4-positive (ADAMTS4+) cells. Investigation of human lung transplant tissues, single-cell and spatial transcriptomic datasets, and functional ex vivo interventions reveal strong clinical relevance. Our study underscores the significance of the lipofibroblast-to-myofibroblast reversible switch in fibrosis development and resolution and identifies ADAM metallopeptidase with thrombospondin type 1 motif 4 as a potential therapeutic target in human lung fibrosis. In the next phase of this study, we are planning to screen an FDA-approved-drugs-library aiming to find a novel inhibitor for ADAMTS4 and test it efficacy in our fibrosis pipeline including mouse and human PCLS as well as lung organoids. Additionally, we will also attempt to unravel the molecular action mechanism ADAMTS4 during fibrogenesis via studying the extra cellular matrix in the lung.

A1-14: Restoration of mesenchymal identity rewires alveolar communication to prevent dysplastic lung repair

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Viral respiratory infections can lead to severe lung injury causing catastrophic loss of the alveolar niche, often triggering dysplastic remodeling instead of functional repair. While the dynamics of epithelial progenitor cells are well characterized, the contribution of the mesenchyme to euplastic regeneration remains elusive. In this study, we reinforced the alveolar fibroblast 1 (AF1) subset of mesenchymal cells to uncover molecular requirements for successful alveolar repair. Using lineage-specific animal models, we demonstrate that the AF1 genetic program is characterized by the activation of a pro-regenerative signaling axis. Single-cell transcriptomics revealed that AF1-derived niche factors target proliferative type 2 alveolar epithelial cells (AT2s), which in turn release sonic hedgehog (SHH) to maintain this mesenchymal niche. We found that this reciprocal mesenchymal-epithelial loop is severely disrupted in both murine influenza models and human ARDS autopsies. Treatment with the recombinant niche ligand accelerates AT2-to-AT1 differentiation, suppresses dysplastic KRT5+ remodeling, and re-establishes intercellular communication. Our data suggest that AF1 dysfunction contributes to pneumonia-associated lung pathology, identify the mesenchymal-HH axis as a critical regenerative engine, and provide a therapeutic roadmap for restoring the alveolar niche following virus-induced lung injury.

A1-15: Y chromosome loss in macrophages promotes inflammatory signaling in age-associated lung diseases

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Mosaic loss of the Y chromosome (mLOY) frequently occurs in aging male leukocytes. Its prevalence increases with age and correlates with increased mortality, especially in cardiovascular and age-related diseases. Chronic inflammation is one of the main clinical manifestations in men with mLOY, although its functional impact on lung disease is still unclear. Macrophages play a central role in linking inflammation to cardiovascular and pulmonary disease, especially with increasing age. Given the unclear role of mLOY in lung disease, we first investigate the prevalence of mLOY in patients with pulmonary hypertension (PH) and chronic obstructive pulmonary disease (COPD) and then the mechanisms by which mLOY in macrophages contributes to the progression of PH and COPD. Droplet digital PCR (ddPCR) analysis of 1,317 COPD patients revealed that 19% had mLOY. Of particular note, COPD patients with mLOY had a higher mortality rate and a higher incidence of myocardial infarction than their non-mLOY counterparts. Mechanistically, loss-of-function experiments of Y-chromosome genes in PBMC macrophages from healthy men show upregulation of interferon (IFN) signaling markers such as IL6, RIG-1 and IRF7, indicating altered regulation of the inflammatory response. Functionally, conditional media of Y chromosome-deficient macrophages reduces the rate of apoptosis in smooth muscle cells, indicating a potential role of mLOY in abnormal vascular remodeling by modulating macrophages. These findings suggest that mLOY may exacerbate lung-associated diseases through inflammatory pathways and resistance to apoptosis. However, further research is needed to elucidate the molecular mechanisms by which mLOY triggers IFN signaling in macrophages and to determine its contribution to lung disease.

A1-16: Endothelial cell plasticity in progressive pulmonary fibrosis: Insights from in-vivo lineage tracing

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Progressive pulmonary fibrosis (PF) is the non-resolving fibrotic phenotype of various interstitial lung diseases (ILDs), such as idiopathic pulmonary fibrosis or Systemic Sclerosis-associated ILD. Beyond widespread lung parenchyma destruction, PF is associated with vascular abnormalities, including heterogeneous capillarization, barrier disruption and endothelial hyper-activation. Increasing attention has focused on endothelial-to-mesenchymal transition (EndoMT) potentially driving vascular dysfunction and expansion of profibrotic cell populations. Here, we aimed to assess the presence and extent of EndoMT in the pathogenesis of lung fibrosis.

The fate of genetically labeled endothelial cells was assessed during PF onset and progression in the bleomycin-induced mouse model (3d vs. 14d) and the Fra-2 transgenic murine model (8w vs. 16w) by flow cytometry, multicolor immunofluorescence staining, single-cell RNA sequencing and electron microscopy. Findings were compared to human lung transplant tissue and pulmonary arteries (PAs).

Overall, the number of pulmonary CD31+ endothelial cells was decreased at the late timepoint in both murine models and in human end stage disease. In contrast, we observed an increase in the number of transitional CD31+SMA+ cells at the early disease stage, which reversed at the later stages in the mice. No increase of transitional CD31+SMA+ cells (%) was apparent in human end-stage tissue samples. On both, murine and human tissue slides, there were no changes in the count of CD31+SMA+ cells as assessed by immunofluorescence staining. Moreover, the human and murine EC populations did not show transcriptional signatures consistent with EndoMT across various scRNA-seq datasets over time. Taken together, our data do not indicate the emergence of a sustained and/or pronounced transitional endothelial population, neither during disease onset nor at later stages, including human end-stage lung fibrosis.

A1-17: Pulmonary Vascular Mural Cells Shape the Distal Alveolar Niche in COPD

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Chronic obstructive pulmonary disease (COPD) is a progressive and incurable disease. Long-term exposure to cigarette smoke (CS) or environmental stressors is a primary etiological factor. COPD is characterised by chronic bronchitis, emphysema, and pulmonary vascular alterations. Although the vascular changes occur early in the disease, it is unclear how COPD-associated vascular remodelling shapes the distal alveolar niche.

We analysed human COPD lungs through histological phenotyping and used an animal model of chronic CS exposure to examine the relationship between pulmonary vascular alterations and emphysema. Comprehensive phenotyping stratified human COPD lungs into two groups: (a) moderate emphysema with extensive vascular remodelling, and (b) severe emphysema with less pronounced vascular remodelling. Preserved distal lung architecture correlated with reduced vascular oxidative and nitrosative stress in muscularised pulmonary vessels. We therefore focused on eliminating oxidative/nitrosative stress sources in Acta2+ cells and investigated novel mechanisms that ameliorate CS-induced emphysema. Mechanistic insights were obtained using ex vivo precision-cut lung slices (PCLS), revealing that reducing stress improved repair in alveolar regions adjacent to lineage-labelled mural vascular cells. Single-cell RNA profiling showed increased expression of extracellular matrix (ECM)-related genes in Acta2+ pulmonary vascular cells from mice with low oxidative/nitrosative stress, animals that showed improved lung function. Similar ECM alterations were validated in human COPD lungs upon deep histological phenotyping. Primary pulmonary vascular smooth muscle cells from repaired mouse lungs or their derived ECM supported alveolar epithelial cell growth and differentiation, suggesting a potent mechanism of lung repair.

These results suggest that the vascular ECM environment influences alveolar repair, revealing a new repair mechanism with therapeutic potential.

A1-18: Extracellular Matrix Remodeling Controls Genomic Integrity and chromatin architecture in Fibroblast Populations

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Rational: The extracellular matrix (ECM) regulates growth, differentiation, and repair. Conversely, pathological ECM remodeling drives diseases like pulmonary fibrosis. How ECM changes dictate gene expression remains elusive. We investigated if the microenvironment of ECM signals the nuclear envelope to promote chromatin remodeling and genomic stress. Method: (A) Human IPF or donor lungs. (B) Three sets of animal studies with ECM remodeling: bleomycin-induced neonatal and adult lung fibrosis; neonatal hyperoxia (85% O_2; HYX)-induced lung injury until P14, with recovery to P70. (C) Murine precision-cut lung slices (PCLS) treated with fibrotic cocktail. (D) De-cellularized fibrotic PCLS (dPCLS) were re-cellularized (rPCLS) with lung fibroblasts. (E) Human lung fibroblasts were treated with fibrotic cocktail. Results: (A) Human IPF lungs showed significant increase in heterochromatin (H3k9me3 and H3k27me3), specifically in fibrotic foci, compared to donor. In particular, CTHRC1$^+$ cells (active fibroblasts), the predominant cell type in fibrotic foci, exhibit DNA damage and senescence. (B) Similarly, bleomycin-treated neonatal and adult lungs revealed increased heterochromatin in mesenchymal cells (FoxF1+). In addition, HYX increased the collagen and decreased the elastic fiber fraction. While the overall heterochromatin level remained unchanged at P14, FoxF1+ cells showed a significant increase. The fibrotic ECM persists until P70, with persistently elevated heterochromatin, particularly in FoxF1$^+$ cells. (C) Likewise, PCLS treated with fibrotic cocktail exhibited a marked increase in heterochromatic. (D) rPCLS showed higher heterochromatin in fibroblast. (E) Human lung fibroblast treated with fibrotic cocktail do not show changes in Histone (EHZ2, EHMT2-G9a, KMT2a) or DNA methylation maker (DNMT1, DNMT3A, DNMT3B). Conclusion: Remodeling of ECM promotes genomic stress and chromatin remodeling in fibroblasts, possibly through mechanical control of epigenetic regulators.

A1-19: The impact of cell-type-specific efferocytosis on the functional properties of alveolar macrophages

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Viral pneumonia and acid aspiration are common causes of acute lung injury (ALI) and can progress to acute respiratory distress syndrome (ARDS). Alveolar macrophages (AMs) are sentinel cells essential for maintaining homeostasis, resolving inflammation, and clearing bacteria. Following lung injury, the restoration of proper gas exchange and the rapid clearance of apoptotic cells (i.e. efferocytosis) are vital. Previous work showed that efferocytosis significantly alters AMs metabolic and functional properties, shaping their plasticity in response to bacteria. This study characterizes AMs functional responses during cell type-specific efferocytosis and its impact on the resolution of inflammation in ALI.

The effect of ingesting apoptotic polymorphonuclear neutrophils (PMNs), alveolar epithelial cells (AECs), or Jurkat T cells on AM secretome and gene expressions were examined. Co-culture systems modeled how efferocytic AMs modulate organoid growth and epithelial interactions. In vivo, the contribution of cell type-specific efferocytic AMs to inflammation was assessed following adoptive transfer in murine models of influenza pneumonia or acid aspiration.

Efferocytosis of distinct apoptotic cargos induces divergent secretory signatures, transcriptional and functional states. In vitro, uptake of apoptotic AECs or Jurkat T cells, but not PMNs, enhanced secretory programs and upregulated genes associated with proliferation, differentiation, and survival, with improved epithelial cell function. In vivo, adoptive transfer of AMs reduced inflammation following acid aspiration and influenza infection. Together, this study emphasizes the importance of understanding the effects of various apoptotic cell cargos on AMs and highlights the potential to harness AMs for therapies in ARDS.

A1-20: Understanding the AT1 Cell in lung homeostasis, injury, and repair

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AT1 cells are crucial for gas exchange, covering 95% of the alveolar surface. Their post-mitotic nature makes them highly vulnerable to injury, limiting regeneration. AT1 function relies on interactions with AT2 cells (adult stem cells), endothelial cells, and the ECM. Despite their importance, AT1 cells are the least understood lung cell type, though recent studies highlight their plasticity. A critical gap is understanding their functional heterogeneity and regulation by the microenvironment, including Notch, integrin, and Hippo signaling pathways.

We aim to identify transcriptionally distinct AT1 subtypes and elucidate how the AT1 niche influences their identity. UMAP analysis revealed seven distinct AT1 subtypes in healthy cells. GO enrichment analysis confirmed specialized functions (e.g., RNA processing, cell adhesion), supporting heterogeneity.

Our developed mouse and human alveolosphere models (expressing AGER, HOPX, KRT8) provide a robust platform to explore AT1-AT2 interactions and the regulation of differentiation and function. These preliminary results suggest functional heterogeneity and a potential role for Notch signalling in injury responses from AT1 cells. Understanding these distinct subtypes and their regulation is essential for developing strategies to promote efficient alveolar regeneration in chronic lung diseases.

A1-21: Clonal Hematopoiesis Instructs Maladaptive Tissue Repair to Promote Fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is a progressive, age-associated interstitial lung disease characterized by the failure of adaptive alveolar repair. While local epithelial-mesenchymal crosstalk is well studied, the role of systemic aging-related cues, such as Clonal Hematopoiesis (CH), and whether it directly regulates tissue remodeling remains unclear. Here, we integrate population genomics, preclinical models, and human lung analyses to examine the role of CH in fibrotic lung disease. In large cohorts, IPF was associated with a distinct CH mutational spectrum enriched for non-DNMT3A variants and for larger mutant clones. In mouse models, hematopoietic mutations exacerbated bleomycin-induced fibrosis and reprogrammed macrophages toward inflammatory, profibrotic states, including expansion of a distinct, injury-responsive SPP1+ population conserved in human disease. CH-associated macrophages were sufficient to directly promote fibroblast activation and alter epithelial differentiation, linking hematopoietic genotype to parenchymal remodeling. Consistently, a CH-derived macrophage transcriptional signature predicted adverse outcomes in independent IPF cohorts. Together, these findings identify clonal hematopoiesis as a systemic regulator of tissue repair and demonstrate that somatic evolution in blood can actively instruct organ remodeling through immune-parenchymal interactions. This framework supports the possibility that disease associated selective pressures may shape clonal architecture with functional consequences for lung health.

AREA 2

A2-1: RBPMS and RBPMS2 Preserve Adult Cardiac Function by Orchestrating Alternative Splicing

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Cardiomyocytes are long-lived, mitochondria-rich cells characterized by high metabolic demands and a limited capacity for renewal. Physiological functions of cardiomyocytes decline and homeostatic replacement of cardiomyocytes is attenuated during aging, making the heart more susceptible to disease processes. Recent studies have highlighted the importance of post-transcriptional gene regulation in the context of age-related cardiac changes, in part mediated by RNA binding protein (RBP)-dependent alternative RNA splicing. My previous work demonstrated that embryonic cardiac-specific deletion of RBPMS and RBPMS2 (RBPMS/2) causes pronounced disruption of the splicing network in embryo-to-postnatal stage transitions, which leads to the formation of defective nuclei and disruption of sarcomere structures, eventually resulting in embryonic lethality 1. Here, we observed reduced expression of RBPMS/2 in cardiomyocytes isolated from geriatric mice. Intriguingly, the reduced expression of RBPMS/2 was accompanied by impaired nuclear integrity, recapitulating aspects of the embryonic RBPMS/2 KO phenotype. Furthermore, we indicated that inactivation of RBPMS/2 in adult cardiomyocytes disrupts nuclear integrity. To determine the extent to which genetic loss of RBPMS/2 mimics aging-associated changes, we performed an in-depth comparison of young adult RBPMS/2 double knockout cardiomyocytes with geriatric wild-type cells. We aim to elucidate how RBPMS/2 safeguard cardiac function in adult stage by coordinating alternative splicing networks.

Reference

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A2-2: Reduction of the RNA binding protein RBPMS prevents asthma, induced by loss of 5-Hydroxymethylcytosine (5-hmC)

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In previous studies we have found that the loss of 5-hydroxymethylcytosine (5hmC) after inactivation of the responsible Tet3 gene in mouse smooth muscle cells (SMC) leads to irregular transcription initiation in highly expressed genes and the production of irregular mRNA transcripts. Such transcripts trigger inflammation in the respiratory system and lead to an asthma-like phenotype in mice. Interestingly, the expression of the RNA-binding and -processing protein RBPMS is greatly increased after inactivation of the Tet3 gene, also in murine asthmatic model and asthmatic patient. RBPMS is found specifically in SMCs and, together with associated regulatory RNAs, plays a role in the control of transcription processes and chromatin changes. Reduced expression of RBPMS attenuates pathological changes in Tet3-mutant lungs through repression of the irregular transcription initiation, and normalizes increased H3K36me3 formation. Moreover, heterozygosity of RBPMS can alleviate asthmatic pathology triggered by Tet3 knockout. The interplay between RBPMS and TET3 regulated transcription fidelity might be a crucial therapeutic target to attenuate lung pathogenesis of asthma disease.

A2-3: DNAJA1-Mediated Control of AP-1 Protein Turnover

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DNAJA1, a member of the HSP40 protein family, is primarily known for its role as a co-chaperone assisting HSP70 in protein folding. However, its broader impact on cellular processes remains largely unexplored. To investigate DNAJA1-dependent cellular changes, we performed proteomic analysis following DNAJA1 knockdown. Our data revealed increased protein levels of several AP-1 transcription factors, including JUN and FOSL1. Complementary transcriptomic analysis showed no corresponding increase in their mRNA levels, suggesting that DNAJA1 regulates AP-1 abundance through post-translational mechanisms. These findings were validated by Western blot and qPCR analyses. Furthermore, cycloheximide chase experiments demonstrated that the elevated AP-1 protein levels result from reduced degradation rates, indicating increased protein stability. AP-1 transcription factors are stress-responsive regulators involved in cellular proliferation, inflammation, and adaptation. Their turnover is controlled by complex pathways, including ubiquitin-mediated proteasomal degradation and autophagy. However, the role of DNAJA1 in regulating AP-1 degradation has not been previously characterized. The primary objective of this project is to elucidate how DNAJA1 controls the stability of AP-1 transcription factors and to determine how this regulation influences AP-1-driven cellular responses under stress conditions.

A2-4: Transcriptional regulation and DNA repair by INO80 control vascular contractility and atherosclerosis

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The INO80 chromatin remodeling complex maintains vascular smooth muscle cell identity and function through dual mechanisms. By partnering with YY1, INO80 sustains Myocardin-driven contractile gene expression, preserving arterial pulse wave propagation and blood pressure regulation. Concurrently, INO80 supports DNA repair-dependent FOXO1 activity, which mitigates oxidative stress and metabolic dysfunction. While loss of Myocardin-mediated contractility disrupts vascular mechanics, the impairment of FOXO1 regulation drives increased atherosclerosis. These findings highlight INO80's critical role in balancing VSMC function and vascular resilience, with distinct pathways governing contractility and disease susceptibility.

A2-5: Smooth muscle protein kinases N (PKN), but not Rho-kinases (ROCK), are essential for myogenic response and DOCA-salt-induced hypertension

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The myogenic response is the ability of small arteries to contract in response to an increased intravascular pressure. It is essential to regulate blood flow and organ perfusion. Evidence has been provided for the activation of vascular smooth muscle GPCRs and of the G12/13/RhoA/ROCK downstream pathways to be essential for this process. ROCK1/ROCK2 are Rho-associated kinases considered important regulators of cardiovascular physiology, including smooth muscle contraction. These functions of ROCK are mainly based on the use of the kinase inhibitors Y-27632 and Fasudil which, however, are not specific inhibitors of ROCK but also inhibit Protein kinases N 1 and 2 (PKN1/2). To determine the specific roles of ROCK1/2 and PKN1/2 in smooth muscle (SM) physiology, we generated SM-specific ROCK1/ROCK2, and PKN1/PKN2 KO mice. Surprisingly, Y-27632 inhibited the myogenic response in SM-ROCK1/2-KO arteries similarly as in control arteries, indicating a ROCK1/2-independent target for Y-27632 in this context. Consistent with this, we found that PKN1/2, but not ROCK1/2, were essential to develop a myogenic response. In vivo, SM-PKN1/2 KO mice showed increased hind limb perfusion in basal conditions. In the experimental DOCA-salt hypertension model, the SM-specific deletion of PKN1/2 but not of ROCK1/2 prevented hypertension, indicating that the increase in vascular tone driving hypertension is mediated by PKN1/2 but not by ROCK1/2. Preliminary results show that PKN1/2 deletion in SM reduces the F/G actin ratio in mesenteric arteries upon pressure, as well as the activation of focal adhesion kinase (FAK), although further studies are ongoing to shed more light onto the underlying molecular mechanisms. Our results suggest that PKN1/2 regulate important processes in smooth muscle previously attributed to ROCK due to the use of non-specific inhibitors.

A2-6: TBK1 mediates chronic NF-κB signalling to promote endothelial inflammation and atherosclerosis

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Atherosclerosis arises preferentially at arterial sites exposed to disturbed flow, where low or oscillatory shear stress provokes endothelial dysfunction. In these regions, endothelial cells activate inflammatory pathways, establishing a pro-atherogenic microenvironment. Central to this response is the transcription factor NF-κB, a master regulator of vascular inflammation that controls adhesion molecule expression and leukocyte recruitment. While canonical IKKa and IKKß are known mediators of NF-κB activation under acute inflammatory stimuli, the mechanisms sustaining chronic NF-κB signaling at sites of disturbed flow remain unclear. Noncanonical kinases such as TANK-binding kinase 1 (TBK1), previously implicated in innate immunity and metabolic inflammation, may represent a mechanotransducing node linking particular flow patterns to persistent endothelial activation. Our work aims to delineate how disturbed flow orchestrate kinase pathways controlling NF-κB activation in endothelial cells. We propose that disturbed flow elicits a biphasic response: a rapid, transient activation of canonical IKKa/IKKß, followed by a delayed but sustained activation of TBK1, which independently maintains NF-κB signaling and inflammatory gene expression. By integrating molecular, biomechanical, and in vivo approaches, this study identifies TBK1 as a pivotal protein kinase driving chronic vascular inflammation under disturbed flow and highlights its potential as a therapeutic target in atherosclerosis.

A2-7: understanding the crosstalk between reg proteins and innate immune cells in cardiac remodeling upon mi

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Heart remodeling comprises left ventricle structural, functional and cellular changes after injury. In myocardial infarction (MI), innate immune cells infiltrate the heart, clear cellular debris and form a collagen scar to keep heart function. Within this heterogeneous inflammatory environment, we focus on the role of the Regenerating islet-derived (REG) proteins. Although being expressed under basal conditions in the GI tract and have antimicrobial and regenerative functions, recent findings by our group identified REG3β as a regulator of macrophage trafficking as well as neutrophil clearance in MI. A genetic inactivation of Reg3β causes increased neutrophil persistence which correlated with impaired left ventricular function and a higher incidence of heart rupture.Although these proteins are detected in the ischemic heart upon MI, their kinetics as well as their cellular and organ origin remain unclear. Here, using the murine model LAD ligation, we characterized the kinetics of REGs after MI showing that all members are present in the ischemic heart with partially overlapping patterns. Sc-RNA sequencing on REG-bound cells showed that, upon MI these proteins mainly bind different macrophage subsets in the heart with one binding to a pro-reparative like macrophage subset. We hypothesized that the different chromosomal location of this member could explain a particularly beneficial role in inflammation resolution. Although being detected in the heart upon MI, we could show that their expression is upregulated in distal organs probably contributing to their presence in the heart after the ischemic insult. Further research will allow for the understanding of the role of the REGs in cardiac remodeling after MI and how their "crosstalk" with immune cells is contributing to inflammation resolution to keep up with cardiac function.

A2-8: ‘’Do not eat me’’ LILRB4 signaling as a novel macrophage immunotherapy in lung cancer

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Lung cancer remains the leading cause of cancer-related mortality worldwide. Although current immunotherapies have improved patient survival, the efficacy is still low. More recently, macrophage-mediated immune evasion, particularly via the upregulation of “do not eat me” signals, has gained increasing attention. The leukocyte immunoglobulin-like receptors (LILRs) are new targets for cancer immunotherapy aimed at tumor-associated macrophages (TAMs). An extensive analysis of the expression profiles of LILRs revealed that among LILRs, LILRB4 is highly expressed in TAMs both in vitro as well as in lung cancer patients. In vitro, TAMs-specific blockage of LILRB4 reduced the expression of tumor-promoting M2 macrophage genes, while it significantly increased cancer cell phagocytosis. No effect was observed on cancer cell proliferation and apoptosis. RNA sequencing identified differential expressed genes in LILRB4 knockdown TAMs, which reveal that silencing of LILRB4 inhibits cell cycle progression of TAMs. This result was confirmed by flow cytometry and correlated with reduced phagocytic activity in the G2/M phase. In addition, LILRB4 blockage on the ex vivo model of human precision-cut lung slices (PCLS) and in in vivo lung cancer models reduced tumor growth and differentially affected the immune cell composition. Finally, cancer cell expressed galectin 8 was identified as a ligand for LILRB4 that mediates its effect on phagocytosis. Collectively, our findings indicate that LILRB4 plays a role in macrophage-specific immune evasion in lung cancer and may be a promising target for lung cancer immunotherapy.

A2-9: Unravelling the role of REG proteins for immune protection against viral pneumonia

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Balanced immune responses are essential during respiratory viral infection: insufficient immunity impairs viral clearance, whereas excessive inflammation causes lung damage. REG (regenerating islet-derived) proteins are secreted C-type lectin-like proteins with established antimicrobial and immunomodulatory functions in the pancreas and intestine, but their role in viral pneumonia is unknown. Our preliminary data identify REG proteins as early responders to viral lung infection. Transcriptomic and histological analyses revealed rapid, transient upregulation of Reg3b and Reg3g in mouse lungs shortly after SARS-CoV-2 or influenza A virus (IAV) infection. Notably, Reg expression also increased in the pancreas and the intestine despite absence of detectable local viral burden, suggesting systemic regulation of Reg gene expression. To enable functional studies, we generated a pan-Reg knockout (RegKO) mouse model lacking all Reg family genes. Under steady-state conditions, RegKO mice showed altered baseline lung immunity, including increased alveolar macrophage abundance. Following IAV infection, RegKO mice developed more severe lung pathology, delayed viral clearance, exaggerated recruitment of myeloid cells, impaired T cell responses, and prolonged chemokine expression compared with wild-type controls. These findings support a protective role for REG proteins in antiviral lung immunity. Ongoing work aims to define how REG proteins regulate baseline pulmonary immune tone, identify their cellular targets and downstream pathways, and evaluate the therapeutic potential of recombinant REG proteins in viral pneumonia models. Together, this study establishes REG proteins as novel regulators of host defense and immunopathology during respiratory viral infection.

A2-10: From Damage to Recovery: Stress-Dependent Maintenance Turnover of Mitochondrial OXPHOS Complexes

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Maintenance of mitochondrial oxidative phosphorylation (OXPHOS) complexes is crucial for sustaining bioenergetic function, especially in differentiated and post-mitotic cells. While de novo assembly pathways have been extensively studied, growing evidence suggests that OXPHOS complexes are continuously maintained through selective turnover and repair processes. Defects in these quality control pathways are associated with human disease, emphasising the importance of coordinated chaperone- and protease-driven maintenance by mitochondrial service factors. However, the physiological triggers that initiate these processes are largely unknown. Here, we combined complexome profiling with pulse-SILAC labelling to investigate how defined mitochondrial stress conditions shape the transition from damage to recovery in OXPHOS complex maintenance turnover. Preliminary data from a human cell line (HEK) were obtained by applying mitochondrial stressors, including transient inhibition of mitochondrial translation, redox stress, and combined OXPHOS inhibition, followed by pulse labelling to detect recovery-related incorporation of newly synthesised proteins within native protein complexes. Different stress conditions produced markedly distinct turnover signatures. Transient inhibition of mitochondrial translation caused a moderate but consistent increase in the incorporation of newly synthesised proteins across multiple OXPHOS complexes, consistent with compensatory recovery-phase replacement. Conversely, combined OXPHOS inhibition resulted in a pronounced loss of complex abundance together with strongly reduced turnover, indicating impaired recovery and suppressed replacement capacity. Under the tested conditions, redox stress caused only minor effects on assembled OXPHOS complexes. Overall, these findings suggest that mitochondrial maintenance is not constant but depends on the type of stress stimulus, thereby defining whether OXPHOS complexes undergo effective recovery.

A2-11: Loss of the triplex-forming lncRNA NTRAS drives vascular inflammation and cardiac dysfunction

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Inflammation drives cardiovascular disease. We identified a CD45^{+} immunomodulatory endothelial cell population emerging in infarct and border zones early after myocardial infarction. Derived from pre-existing endothelial cells, these cells express MHC-II and RUNX1 and promote immune cell recruitment and activation via pro-inflammatory cytokines. Long non-coding RNAs (lncRNAs) control cellular functions and represent promising therapeutic targets to modulate cell plasticity. Notably, we found that deficiency of the lncRNA Ntras induces cardiac inflammation in mice. Given its strong impact on endothelial behavior, we hypothesize that Ntras modulates endothelial-immune communication after infarction. In vitro, cytokine-driven induction of an immunomodulatory EC phenotype led to a marked and robust downregulation of NTRAS expression. Conversely, NTRAS silencing was sufficient to induce an IMEC-like switch of ECs reflected in an enhanced expression of adhesion molecules and pro-inflammatory signaling. RNA-sequencing of ECs from Ntras-deficient mice revealed enhanced expression of well-known adhesion and antigen presentation genes, mirroring the immunomodulatory EC signature and correlating with systolic and diastolic dysfunction. The nuclear localization of NTRAS, together with the well-known role of lncRNAs in RNA:DNA:DNA triplex formation, led us to investigate whether NTRAS regulates endothelial plasticity through a triplex forming region (TFR). Computational analyses identified multiple candidate target genes containing potential triplex-binding sites, including the anti-inflammatory and pro-angiogenic G protein subunit alpha i2 (GNAI2) and RUNX1, in both human and murine ECs. Deletion of the NTRAS TFR in vitro and vivo recapitulated immune-vascular remodeling and progressive cardiac dysfunction. Our findings identify the NTRAS TFR as a key regulator of triplex target genes that connects chromatin interactions with pro-inflammatory signaling in cardiac remodeling.

A2-12: Using pairwise RPG co-expression to quantify ribosomal heterogeneity at single cell resolution

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Ribosomal protein genes (RPGs) are conventionally regarded as stably expressed housekeeping genes encoding structurally uniform ribosomes. Mounting evidence challenges this view: ribosome composition varies across cell types and conditions, with distinct ribosomal protein stoichiometries preferentially translating functionally coherent mRNA subsets. However, methods to quantify ribosomal heterogeneity in primary human tissue have been lacking. Here we present RPGFingerprints, a computational framework that clusters cells by pairwise RPG co-expression patterns in single-cell RNA sequencing data. RPG-based clustering in PBMC achieved a mean cell type purity of 83.6%, with cell types organized orthogonally to conventional HVG-based transcriptomic projections. Applied to $1{,}468{,}300$ cells from 310 donors across two tissues and two disease contexts, RPGFingerprints resolves discrete ribosomal states which associate with disease status, severity, and clinical outcome. In COVID-19 monocytes, RPG stoichiometry alone identifies a recovery-associated state marked by wound healing and inflammatory resolution signatures as well as a fatality-associated foam-cell-like state, recapitulating known disease biology without further transcriptomic or clinical input. Transcription factor activity analysis further provides mechanistic insight linking RPG stoichiometry to selective translational preferences, offering population-scale in vivo support for the specialized ribosome hypothesis. RPGFingerprints is released as an R package.

A2-13: Not so NEATly non-coding: a novel nuclear peptidein encoded by the lncRNA NEAT1 modulates DNA replication and cell proliferation

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Peptidein encoded by long non-coding RNAs (lncRNAs) are an emerging class of molecules with potential roles in vascular biology. However, their function remains largely unknown. This study focused on the characterization of a 71 amino acid peptidein encoded by the lncRNA NEAT1 in human endothelial cells, i.e. pep-NEAT1.

In endothelial cells expressing a FLAG-tagged pep-NEAT1 fusion protein, pep-NEAT1 localized to the nucleus (FLAG immunofluorescence) and elicited the disruption of promyelocytic leukaemia nuclear bodies, suggesting a possible involvement in the regulation of gene expression. FLAG-pep-NEAT1 immunoprecipitates from nuclear endothelial cell extracts were analysed by mass spectrometry to identify proteins interacting with the peptidein. This analysis identified interactions with proteins involved in DNA replication, including the DNA helicase complex, and nucleotide metabolism. As a consequence, in pep-NEAT1-expressing cells, genes related to cell proliferation, migration and angiogenesis were significantly downregulated, while genes associated with inhibition of cell growth were upregulated. Consistent with these alterations, pep-NEAT1-expressing endothelial cells exhibited reduced cell proliferation and migration.

Altogether, these results suggest that endothelial pep-NEAT1 is a novel nuclear peptidein involved in the regulation of fundamental processes, including nuclear body organization, DNA replication, and cell proliferation.

A2-14: The biallelic lncRNA LINC00106 couples hnRNPK binding to Endothelial Dynamics In Cardiovascular Regulation

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Precise subcellular RNA localization is a key determinant of RNA function, influencing diverse cellular processes. RNA localization can be determined by various factors, one of which is splicing. Intron retention (IR), an alternative splicing event, plays a regulatory role in RNA localization by promoting nuclear retention of the transcripts, acting as a translational buffer, or facilitating compartment-specific structural functions needed for cellular processes. Cellular stress such as hypoxia can often alter splicing patterns. CVDs are associated with low oxygen availability(hypoxia), where cells undergo significant changes in gene expression and RNA processing to promote survival. However, the role of IR in RNA dynamics and gene regulation in hypoxia and CVDs remains poorly understood. Our results indicate that IR is the most frequently altered splicing event under hypoxia, inducing persistent alterations in splicing,accompanied by significant changes in RNA subcellular localization that are maintained during reoxygenation,with IR-RNAs accumulating at nuclear speckles, indicating a spatial regulatory layer in RNA processing. While hypoxia is known to affect RNA metabolism, our findings indicate that reoxygenation further reinforces these effects, leading to sustained changes in splicing, localization, and gene expression. Our work identify an lncRNA called LINC00106, which is a biallelic, hypoxia-responsive IR-RNA,essential for key endothelial functional phenotypes. LINC00106 is predominantly nuclear and functions in trans, with strong affinity to hnRNPK binding and triplex-forming potential, suggesting that it mediates gene regulation through specific RNA–protein and RNA–chromatin interactions. By integrating advanced imaging, transcriptomics, and computational analyses, this project aims to define the molecular mechanisms of IR-LINC00106 and its role in hypoxia-driven endothelial adaptation, with potential implications for novel therapeutic strategies in CVDs.

A2-15: Prediction of Split Open Reading Frames across cell types

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Background: Split Open Reading frames (Split-ORFs) exist on transcripts containing at least two open reading frames, each of which encodes a part of the same full-length protein. These multiple open reading frames arise from alternatively spliced transcript isoforms. The phenomenon of Split-ORFs has been observed for the SR protein family of splicing factors, where the Split-ORF proteins play important autoregulatory roles.

Aims/purpose: OurThe aim of this study wasis to investigate the expression translation and expression translation of Split-ORFs.[MS1.1] by predicting them for a set of transcripts and validating their presence with multimodal experimental data.

Methods: We built a pipeline that predicts potential Split-ORFs for a user supplied set of transcripts and determines the regions unique to the potential Split-ORFs. These unique regions are absent from protein coding transcripts. The translation of the predicted Split-ORFs can be validated by finding their unique regions in Ribo-seq or proteomics data.

Results: The Split-ORF pipeline was applied to a set of transcripts containing premature termination codons or retained introns. Novel Split-ORF transcripts and their unique regions were predicted and a substantial fraction had significant Ribo-seq coverage in data of from different cell types. Additionally, the Split-ORF candidate start sites had a significantly higher probability of being translation initiation sites than background sites as predicted by a deep neural network.

Outlook: These results suggest that Split-ORFs the occurrence of Split-ORFs is more occur more widespread than previously assumed and that they are expressed across different cell types. This paves the road for further functional investigations of the validated Split-ORF candidates and mechanisms of their biogenesis.

A2-16: Actin cytoskeleton rewires nuclear architecture in pulmonary hypertension via Hippo/RhoA axis

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PH is a fatal disease with multifactoral pathology. Current therapies are limited, highlighting the need to identify novel, druggable signalling axes. Because PH is driven by epigenetic regulators and transcription factors, understanding how nuclear architecture and corresponding chromatin organization are altered in PH is crucial yet remains poorly explored.

In this study, we investigated nuclear alterations in PH, their links to chromatin remodelling and associated signalling pathways.

Imaging revealed marked differences in nuclear structure between healthy individuals and PH patients. Multi-omics analysis identified dysregulation of nucleoproteins, altered chromatin states, and signalling pathways associated with these nuclear changes. Functional studies showed that SUN1 accumulation and Lamin A/C loss mimics PH signatures.

Pathway enrichment indicated that actin cytoskeleton remodelling was a major driver of these phenotypes. In vitro, actin activation via Jasplakinolide treatment or culture on stiff matrices reproduced the nuclear and chromatin changes observed in patient samples. RNA sequencing of Jasplakinolide-treated cells highlighted the Hippo pathway as an upstream mediator of actin remodelling. Furthermore, pharmacological inhibition of Hippo signalling reversed actin remodelling, nuclear architectural alterations, and chromatin dysregulation across in vitro, ex vivo, and in vivo PH models.

Collectively, our findings identify nuclear architectural disruption as a hallmark associated with PH.

A2-17: Impaired antioxidant defense increases susceptibility to pathological pulmonary vascular remodeling upon COPD-relevant exposure

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Pulmonary vascular alterations occur early in chronic obstructive pulmonary disease (COPD) and may contribute to emphysema pathogenesis. Oxidative/nitrosative stress is a major driver of COPD and involves impaired heme oxygenase-1 (HO-1) antioxidant defense. Such stress leads to inflammation and pulmonary vascular alterations. In COPD, the role of HO-1 has primarily been investigated in immune cells; its function in non-immune cells, which may require it even more, remains understudied.

Here, we exposed conditionally HO-1-deficient mice and controls to chronic cigarette smoke (CS). The pulmonary vascular phenotype was assessed by right heart catheterization and echocardiography. Lungs and bronchoalveolar lavage fluid were collected for histological and flow cytometric analyses. Furthermore, to investigate the specific contribution of the non-immune compartment to the defense against CS injury, lavaged lungs were used to generate ex vivo precision-cut lung slices (PCLS).

Upon CS exposure, HO-1-deficient mice showed increased right ventricular systolic pressure, reduced tricuspid annular plane systolic excursion, and increased pulmonary vascular muscularization, compared to controls. Deletion of HO-1 elevated infiltrating interstitial macrophage, monocyte, and CD4+/CD8+ T cell numbers after CS exposure, whereas alveolar macrophage numbers were decreased. Ex vivo, loss of HO-1 in PCLS, which are depleted of alveolar macrophages and lack recruited infiltrating macrophages, reduced metabolic activity and upregulated cytotoxicity and apoptosis markers upon CS injury, compared to intact control PCLS.

These findings show that non-immune HO-1 is crucial for limiting CS-induced cytotoxicity, inflammation, and early vascular alterations. Future studies will identify the specific cellular drivers of this remodeling and utilize long-term CS models to assess how impaired HO-1 defense impacts emphysema development.

A2-18: HDAC9 in COPD: An Epigenetic Regulator of Lipid-Driven Metainflammation

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Aging is a major risk factor for chronic lung diseases such as COPD, yet the molecular drivers linking aging to disease susceptibility remain poorly defined. Among class IIa histone deacetylases, HDAC9 emerged as the only member consistently reduced in lungs from elderly versus young donors, suggesting a unique role in lung aging. To investigate its relevance in COPD, we analyzed genetic, lipidomic, and functional consequences of HDAC9 loss in human samples and mouse models. In early-stage COPD patients (n = 236), a loss-of-function HDAC9 SNP was associated with lower lung function (FEV_1, DLCO) and elevated systemic inflammatory markers, including IL-6 and TNFα. In serum from SNP carriers, total choline-containing phospholipids were inversely associated with FEV_1, indicating that higher phospholipid levels accompany worse airflow limitation. Lipidomic profiling further revealed broad redistribution of circulating lipid classes, including depletion of major phospholipid pools, supporting a genotype-dependent remodeling of the pro-inflammatory lipid milieu. Consistent with these findings, Hdac9-deficient mice showed altered phospholipid metabolism, premature alveolar damage, loss of alveolar epithelial cells, and early senescence. With aging, Hdac9-/- lungs developed enhanced $CD4^+$ and $CD8^+$ T-cell accumulation, inflammation, and vascular remodeling. Importantly, restoration of HDAC9 expression ameliorated the COPD-like phenotype, including emphysema-like structural abnormalities. Moreover, senolytic treatment improved alveolar architecture and reduced inflammation, functionally confirming epithelial-intrinsic senescence in Hdac9-deficient lungs. Together, these findings identify HDAC9 loss as a driver of epithelial senescence, metabolic dysfunction, and progressive lung injury during aging, and nominate HDAC9 as a biomarker and potential therapeutic target in early COPD.

A2-19: Spatial immune ecosystems define outcome of lung cancer immunotherapy

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Immunotherapies have significantly transformed cancer treatment, especially for lung cancer. However, only about 20% of lung cancer patients respond positively to these therapies. The spatial arrangement of immune cells within tumors is crucial for understanding the intercellular interactions that influence clinical outcomes. Therefore, it is important to investigate the spatial distribution and cellular interactions in NSCLC patients who later receive PD1 inhibitor-based immunotherapy. In a study of 30 NSCLC patients treated with the PD1 inhibitors Nivolumab and Pembrolizumab, with or without preoperative therapy, tumors or biopsy tissues were collected to analyze spatial characteristics in 17 responders and 13 non-responders. Tissue sections from FFPE lung tumors underwent high-plex immunofluorescence targeting 22 biomarkers, including structural, vascular, immune (myeloid and lymphoid cell phenotypes), and functional markers. After cell segmentation, we analyzed cell phenotype distribution based on cell-type-specific marker expression using refined analysis algorithms on the CeltixTM image analysis platform. Good responders showed an immune-enriched tumor microenvironment with increased B cells, monocytes, and T helper cells, and reduced tumor proliferation, while non-responders exhibited higher cytotoxic T cell infiltration and more proliferating tumor cells. Preoperative treatment caused significant remodeling, increasing dendritic cells, Tregs, T helper cells, endothelial cells, and M2 macrophages, while reducing proliferating tumor cells and M1 macrophages. Spatial analysis showed that responders had tighter coordination among immune cells, while treatment increased separation between proliferating tumor cells and immune populations but enhanced proximity to apoptotic tumor regions. Cellular neighborhood analysis identified ten distinct niches, including tumor, immune-active, and immunosuppressive states. Responders were enriched in immune-active neighborhood

A2-20: HyPeLi, a peptide-encoding lncRNA, orchestrates cellular adaptation under hypoxia and in chronic lung diseases

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Hypoxia, defined as insufficient oxygen levels, drives various pathological processes in the lungs. Beyond protein-coding genes, lncRNAs are emerging as key regulators of hypoxia-responsive pathways. In this project, we investigate the role of a novel lncRNA, HyPeLi, which exhibits high coding potential, in hypoxia signaling and chronic lung disease pathogenesis. HyPeLi expression in hPASMCs showed a time-dependent increase under hypoxia, peaking at 24hrs and declining thereafter. This temporal expression pattern closely paralleled apoptosis during the acute phase of hypoxia in hPASMCs. Functional modulation of HyPeLi demonstrated that it induces apoptosis under hypoxia. Interestingly, in silico analyses predicted that HyPeLi has coding potential, suggesting possible translation into a peptide. This previously unidentified peptide was confirmed using a custom antibody targeting the HyPeLi-derived sequence. Subcellular localization studies showed that both HyPeLi RNA and its peptide are present in nuclear and cytoplasmic compartments. Transcriptomic profiling revealed that HyPeLi regulates pathways involved in cellular stress response, cell cycle control, and protein translation during hypoxia. In disease context, HyPeLi RNA was upregulated in PH and IPF, whereas its encoded peptide was reduced, indicating potential post-transcriptional regulation and contribution to the observed apoptosis resistance in these disease conditions. To assess its therapeutic potential, recombinant HyPeLi peptide was applied to in vitro and ex vivo models of PH and IPF, where it induced apoptosis and reversed disease phenotypes. Together, these findings identify HyPeLi as a novel regulator of hypoxia-driven cellular adaptation and highlight its therapeutic potential in chronic lung diseases.

A2-21: Mitochondrial Ribosomal Protein MRPL3 in lung cancer progression

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Lung cancer remains the leading cause of cancer-related mortality worldwide, highlighting the urgent need for novel molecular targets beyond current targeted and immune-based therapies. Mitochondrial reprogramming has emerged as a key driver of tumor progression and therapy resistance, yet the contribution of mitochondrial ribosomal proteins (MRPs) to lung cancer biology is not completely understood yet. Among these, MRPL3 is essential for the translation of mitochondrially encoded electron transport chain components and the maintenance of oxidative phosphorylation (OXPHOS). Recent pan-cancer analyses have identified MRPL3 as significantly upregulated in multiple malignancies, including gastric and pancreatic cancer. Notably, high MRPL3 expression correlates negatively with immunogenic markers, and immune cell infiltration suggesting a potential role in shaping an immunosuppressive tumor microenvironment (TME). However, its functional role in lung cancer remains largely undefined. In tumor cells, we have shown that increased MRPL3 expression supports proliferation, redox homeostasis, and resistance to apoptosis. In parallel, these cells influence the polarization and activity of tumor-associated macrophages, which rely on distinct metabolic programs to adopt pro- or anti-tumorigenic phenotypes. Targeting MRPL3-dependent pathways may provide a new strategy to impair tumor metabolic flexibility and overcome immune evasion in lung cancer.

A2-22: Mitochondrial Cox4i2 drives pulmonary inflammation and emphysema

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Chronic obstructive pulmonary disease (COPD) is characterized by persistent neutrophilic inflammation, progressive emphysema, and the development of mild pulmonary hypertension (PH). A growing body of evidence indicates that oxidative and nitrosative stress play a central role in disease initiation and progression; however, the specific cellular and molecular mechanisms driving these processes in distinct lung cell populations remain poorly understood. We demonstrate that expression of mitochondrial cytochrome c oxidase subunit 4 isoform 2 (COX4I2), a subunit of respiratory chain complex IV, increases after three months of cigarette smoke (CS) exposure in mice and in early human COPD. Global Cox4i2−/− mice are protected from CS-induced emphysema but not PH, and this protection is associated with reduced nitrosative stress, inflammation, and apoptosis.

To identify the relevant cellular sources of COX4I2, we employed a novel Cox4i2 reporter mouse model together with in situ hybridization analyses of human lung samples. These approaches revealed that COX4I2 is predominantly expressed in $ACTA2^{+}$ precapillary smooth muscle cells and capillary pericytes. Mechanistically, COX4I2 promotes mitochondrial reactive oxygen species (mtROS) production in these cells, enhancing alveolar type II cell apoptosis and neutrophil migration, respectively.

In contrast to genetic deletion of Cox4i2, treatment with the mitochondria-targeted antioxidant MitoQ effectively reversed both emphysema and PH in CS-exposed mice. Collectively, these findings suggest that COPD pathologies are regulated in a cell-type–specific manner and identify mtROS signaling as a promising therapeutic target for COPD.

AREA 3

A3-1: Cell-specific transcriptomics identify cross-communication networks between endocardial cells and cardiomyocytes during zebrafish heart regeneration

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Unlike the adult mammalian heart, the adult zebrafish heart fully regenerates post-injury. This unusual regenerative capacity relies on the developmental competence of its mature cardiomyocytes (CMs) and their responsiveness to well-orchestrated, paracrine inputs from non-myocytes. Among the different non-myocyte populations, endocardial cells (EdCs), which ensheathe the myocardium have emerged as key regulators of CM renewal and regeneration. However, comprehensive datasets providing cell-to-cell resolution of stage-specific, injury-activated ligand-receptor interaction networks between these cells are lacking.

Here, we resolved transcriptomic profiles of EdCs and CMs, sorted from remote and injury-border zone areas following ventricular cryoinjury. Both cell-types activated robust immunogenic, neurogenic, proliferative and angiogenic responses within 24-48 hours. Notably, the previously reported MHC II antigen presentation genes that peak in EdCs between 7-14 days, spiked in CMs at 48 hours, suggesting early involvement of CMs in innate-to-adaptive immune regulation. We reconstructed >30,000 bidirectional and endocardial-to-myocardial interactions between 657 injury-upregulated paracrine factors and 434 receptors, and identified >250 transcription factors. Integration of these data with our lab's DanioHeart atlas, further identified >700 developmental factors re-expressed upon injury. In addition, 270 orthologous genes were found downregulated in the regeneration-incompetent postnatal-day-8 (P8) mouse hearts, compared to the regenerative P1 stage hearts.

Altogether, our work defines conserved paracrine factors and signaling networks in zebrafish that may be leveraged in adult mammals to enhance cardiac regeneration.

A3-2: Intersectional genetics approach (ISGA) enhances the precision of de-differentiated cardiomyocyte lineage tracing and manipulation

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Irreversible cardiac fibrosis, cardiomyocyte (CM) loss, and chronic dysfunction following myocardial infarction remain major clinical challenges with no curative therapies. Adult mammalian CMs exhibit minimal proliferative capacity, limiting endogenous heart regeneration. CM dedifferentiation is a prerequisite for proliferation, characterized by reactivation of developmental programs and structural remodeling, yet remains poorly defined due to the lack of specific molecular markers and robust in vivo tools. To address this limitation, we establish an intersectional genetic approach (ISGA) that enables selective labeling of immature or dedifferentiated CMs using dual recombinase systems driven by Tnni3 and ACTA2 promoters. This system provides precise genetic access to a previously inaccessible cardiomyocyte subset and its transitional states, facilitating targeted manipulation of candidate genes to enhance regional regeneration and minimize scar formation.

A3-3: Spatial Mapping of Metabolic Remodeling in Lung Disease

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Cellular metabolism undergoes dynamic reprogramming across a wide spectrum of lung diseases, including cancer, chronic obstructive pulmonary disease (COPD), pulmonary hypertension (PH), and fibrosis; however, its spatial organization and evolution during disease progression remain incompletely understood. Here, we performed untargeted spatial metabolomics and lipidomics using MALDI mass spectrometry imaging on whole-lung sections from wildtype controls and KrasLA2 mice, a model of spontaneous lung tumor development representing disease-associated metabolic remodeling.

Unsupervised segmentation of combined metabolite and lipid profiles delineated distinct tissue regions, including normal lung and multiple disease-associated subregions. These metabolically defined areas showed strong concordance with histopathology and enabled classification of lesions by size and progression stage. To identify discriminative features, we applied receiver operating characteristic (ROC) analysis across regions and disease stages. Ranking annotated metabolites and lipids by ROC values and directionality revealed consistent molecular clusters associated with pathological remodeling.

Tumor regions exhibited increased levels of glucose-1-phosphate and tricarboxylic acid intermediates, which further accumulated from early to advanced tumor development. Lipidomic analysis identified coordinated enrichment of polyunsaturated phosphatidylethanolamines, phosphatidylcholines, and phosphatidylinositol-related species, while sphingomyelins were relatively higher in normal and early-stage regions. Notably, the number of discriminative features decreased along progression, indicating early metabolic remodeling followed by selective stabilization.

Together, these findings define a spatial metabolic trajectory of lung disease and highlight stage-specific metabolic signatures with potential relevance for biomarker discovery and therapeutic targeting across diverse pulmonary pathologies.

A3-4: Truncating mutations in cardiac myosin heavy chain isoforms lead to genetic compensation in zebrafish

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MYH6 and MYH7 are paralogous myosin heavy chain genes located in a conserved tandem gene cluster in mammalian genomes. Gain-of-function mutations in MYH7 are a leading cause of hypertrophic cardiomyopathy (HCM), whereas truncating variants that introduce a premature termination codon are found in apparently healthy individuals. However, recent studies have reported truncating variants associated with HCM. As cardiac myosin isoform expression is reversed in the postnatal mouse heart, we examined the expression and function of cardiac myosin isoforms in the zebrafish model. Here, we report that zebrafish undergo a vmhc-to-vmhcl isoform switch in ventricular cardiomyocytes during development, orthologous to the MYH6-to-MYH7 isoform switch observed in developing mammalian hearts. We found that this switch is completed in trabeculae cardiomyocytes earlier than in compact layer cardiomyocytes but that it is not reversed in zebrafish. Characterisation of in-frame mutations in vmhc and vmhcl identified temporally distinct and fully penetrant recessive silent ventricle phenotypes. However, truncating mutations resulted in mild and incompletely penetrant phenotypes in homozygous mutants that are viable and fertile. In-frame mutations were associated with mild upregulation of the mutant gene, whereas truncating mutations were associated with low expression of the mutant gene, indicative of mutant mRNA decay by the nonsense-mediated mRNA decay pathway. These results are consistent with genetic compensation via transcriptional adaptation, whereby mRNA decay upregulates the expression of related genes based on sequence similarity. Together, these results indicate that zebrafish accurately model cardiac myosin isoform expression and suggest that truncating variants in cardiac myosin heavy chain isoforms may contribute to disease through dysregulation of related genes as a result of transcriptional adaptation.

A3-5: Investigating the onset of cardiac fibrosis in a novel zebrafish model

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Cardiac fibrosis (CF) is a pathophysiological condition associated with many types of cardiovascular disease. CF is characterized by excessive collagen deposition in the extracellular matrix (ECM), driven by increased differentiation of cardiac fibroblasts into myofibroblasts in response to tissue stress. Despite its clinical importance, the mechanisms underlying the onset and progression of CF remain poorly understood. As existing models to study CF suffer from substantial limitations including ethical considerations, high maintenance costs, and inability to perform live imaging at cellular resolution, we have developed a novel gain-of-function zebrafish model that exhibits increased collagen deposition within the atrial tissue starting by 1-month post-fertilization, followed by its resolution by 6 months. In this model, continuous overexpression of the voltage-gated sodium channel gene scn5lab in atrial cardiomyocytes causes an increased heart rate. These transgenic zebrafish display impaired cardiac function as evidenced by reduced fractional shortening, absent/short P-waves, and disrupted calcium handling. They also exhibit increased blood regurgitation as well as atrial enlargement, possibly a consequence of ECM remodeling, as suggested by the dysregulation of ECM related genes. To investigate the early events leading to CF, we performed scRNAseq at 1 month, which allowed us to capture all cardiac populations present in the atrium. This dataset revealed the increase of different cell types (e.g., macrophages and fibroblasts) during the initiation of CF. Specifically, we identified two cardiac fibroblast subtypes that were increased in the scn5labGOF and may contribute to CF. Comparative transcriptomics between fibrotic and control conditions identifies many ECM-related genes as well as several transcription factor genes that may play an important role in CF. These findings were further validated by in vitro experiments involving human cardiac fibrobl

A3-6: The role of TFPT in a INO80-dependent manner during heart development

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The INO80 chromatin remodeler complex regulates transcription and chromosome maintenance through interactions with transcription factors and non-coding RNAs. It comprises eight conserved core subunits and six metazoan-specific subunits, including TFPT, which has been identified as a fusion partner of transcription factor 3 in childhood leukemia and is predicted to have both DNA and kinase binding activities. This study investigates the molecular basis and cell-of-origin of fetal lethality caused by TFPT deletion.

We found that TFPT$^{-/-}$ embryos exhibit lethality beginning at E11.5, with prominent cardiovascular defects, notably ventricular septal defects. Transcriptomic analysis revealed downregulation of chamber morphogenesis genes and this finding was supported by the histological phenotypes including retarded ballooning and poor ventricular compaction. Co-immunoprecipitation confirmed that TFPT is essential for INO80 complex stability. Its loss triggers significant compositional shifts within the complex. Furthermore, ChIP-seq analysis revealed that TFPT's genomic recruitment is mediated by the canonical INO80 complex and is specifically dependent on its interaction with the transcription factor YY1. Single-cell RNA sequencing identified a marked reduction in epicardium-derived fibroblasts in TFPT$^{-/-}$ hearts. By combining the RNA-seq and ChIP-seq data, we propose that TFPT directly regulates the transcription of A-Raf and C-Raf, and that its absence leads to a failure in the Raf-MAPK signaling pathway during the epithelial mesenchymal transition of epicardium. Together, these findings identify TFPT as a crucial subunit for the recruitment of YY1 into INO80 complex and provide a mechanistic link between chromatin remodeling and lineage-specific signaling cascades during cardiogenesis.

A3-7: Regulation of Bronchioalveolar Stem Cells in Health and Disease

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Lung disease remains a major global health burden, causing high morbidity, mortality, and economic loss. Understanding lung regeneration and stem cell regulation at cellular and molecular levels is essential for advancing repair therapies. Niche-associated, marker co-expressing bronchioalveolar stem cells (BASCs) contribute substantially to distal lung regeneration by differentiating into Club or alveolar type 2 cells, but the molecular mechanisms controlling BASC proliferation and differentiation remain unclear.

To identify key mechanisms directing lineage specification of BASCs, we performed bulk RNA sequencing of FACS-isolated BASCs under steady-state conditions and after bronchiolar or alveolar injury, focusing on their primed state at an early time point following injury. Candidate signalling pathways were validated through a combination of in vitro and in vivo approaches, including 3D organoid cultures and mouse models with BASC-specific loss- and gain-of-function mutations. We found that Notch and Wnt/β-catenin signalling are differentially regulated in BASCs after injury. Notch activation drives secretory differentiation while inhibiting alveolar cell fate, whereas its loss promotes alveolar differentiation. Conversely, Wnt/β-catenin activation stimulates BASC expansion and alveolar differentiation while suppressing secretory lineage commitment; β-catenin loss reverses these effects and depletes the BASC population.

We conclude that BASC differentiation and expansion is governed by the tight interplay between Notch and Wnt/β-catenin signalling, uncovering new regulatory mechanisms of lung epithelial regeneration.

A3-8: Factor XIII+ macrophages promote coronary revascularization and cardiac regeneration in adult zebrafish

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The modulation of macrophage states is critical for successful regeneration, highlighting the importance of analyzing the function of all macrophage populations. Using single-cell profiling of mpeg1+ macrophages during zebrafish cardiac regeneration, we identified 10 different populations with distinct dynamics. We focused on a distinct pro-regenerative population expressing factor 13 (f13a1a.1). Using newly generated lines, we first found that f13a1a.1+ cells localize adjacent to coronary endothelial cells (cECs) around the injured area, and that ablation of f13a1a.1+ cells led to a significant reduction of cEC-associated macrophages and reduced cEC proliferation. Upon cardiac cryoinjury, f13a1a.1 mutants exhibit altered macrophage populations, defective coronary revascularization, and cardiac regeneration failure. Macrophage-specific overexpression of wild-type and dominant-negative f13a1a.1 affected multiple macrophage populations, indicating a fine balance between them. Pharmacological rescue experiments indicate that F13a1a.1 promotes cEC proliferation through pERK activation and Thrombospondin-1 inhibition. Collectively, these findings identify f13a1a.1+ macrophages as key regulators of cEC revascularization and further reveal the multifaceted functions of macrophages during cardiac regeneration.

A3-9: Modifying the Code: RNA-Modifying Enzymes as Architects of Embryonic Translation

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RNA modifications expand the informational capacity of the transcriptome beyond sequence, yet how this layer is interpreted to control gene expression remains largely unresolved. Although more than 170 RNA modifications have been identified, their dynamic deployment during development and their functional integration with the translational machinery remain poorly defined. Emerging evidence suggests that RNA modifications regulate mRNA stability and translation, but whether they act as an instructive system that programs cell fate decisions is unknown, revealing a fundamental gap in developmental gene regulation. Rather than acting as static marks, RNA modifications may function as a programmable regulatory layer interpreted at the ribosome to selectively direct protein synthesis. Supporting this concept, we have identified selective interactions between RNA-modifying enzymes and actively translating ribosomes during human pluripotent stem cell differentiation, pointing to a regulatory axis at the ribosome itself. We hypothesize that ribosome-associated RNA-modifying enzymes serve as modular regulators of cell fate by coupling chemical state of mRNAs to their translational output. Using cardiac development as a model, this project will define how RNA modification landscapes are dynamically remodeled during cell fate transitions, how RNA-modifying enzymes interface with RNA-binding proteins and ribosomes, and how these interactions control mRNA stability and translation. Our central objective is to uncover how the ribosome interprets chemically modified transcripts to shape developmental gene expression programs. By establishing a mechanistic framework that links RNA modification identity to translational control and cell fate, this work positions the epitranscriptome as a central regulatory axis of development. More broadly, it will redefine gene regulation by revealing how chemical modifications of RNA encode functional specificity at the level of protein synthesis.

A3-10: Decoding RNA spatial logic during cardiac development

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RNA carries not only sequence information, but also spatial information. Across development, transcripts are selectively positioned within cells to determine where proteins are made, where RNAs are stored, and where they are stabilized or cleared. This is not a rare phenomenon: genome-scale studies in early embryos have shown that subcellular RNA localization is pervasive, yet the molecular rules that establish these patterns and their functional consequences remain poorly defined. In the heart, sarcomeric and structural RNAs are distributed to specific intracellular regions, and microtubule- and kinesin-dependent transport is required to position mRNAs, ribosomes, and local translation at sites of growth. When this spatial organization is lost, translation still occurs, but in the wrong place, and newly synthesized proteins are destabilized, leading to developmental failure. Using cardiac development as a model, this project will define RNA localization as a regulatory layer that actively instructs cardiac development by linking subcellular transcript positioning to RNA stability, translational output, and tissue morphogenesis. Our goal is to define the molecular logic that governs RNA subcellular localization during cardiac development and to determine how spatial transcript organization instructs developmental gene expression. The fundamental question we aim to address here is: How does subcellular transcript localization instruct RNA fate, translation, and cell identity? By combining spatial transcriptomics, including Multiplexed error-robust fluorescence in situ hybridization (MERFISH), perturbation genetics, and synthetic engineering of localization motifs, we will uncover how RNAs encoding determinants of embryonic cell fate and function are targeted to specific subcellular compartments, why this positioning matters, and how it controls RNA stability, translational output, and cardiomyocyte function. This work will establish RNA localization as a fundamental

A3-11: Interleukin-33 and cMAF regulates lymphatic function in cardiac aging

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As life expectancy increases, age-related diseases particularly cardiovascular diseases have become leading causes of death. Aging is the primary risk factor for cardiovascular dysfunction, making it essential to understand the underlying mechanisms. While the microcirculation has been extensively studied, the role of the lymphatic vasculature in age-related cardiac dysfunction remains unclear. Therefore, we investigated the impact of aging on lymphatic function.

We found that lymphatic vessel density declines beginning at 16 months of age, with significant reductions at 18 months in both sub-endocardial and epicardial regions in both sexes. To explore underlying mechanisms, we performed single-cell RNA sequencing on CD31+ endothelial cells from young (3 months) and aged (20 months) mice. Clustering identified a lymphatic endothelial cell population enriched for Prox1 and Lyve1. Aging led to differential expression of 197 genes, including significant upregulation of Il33 and Maf.

Histological analyses confirmed increased IL-33 expression in aged LYVE1+ lymphatics starting at 16 months. Notably, only the nuclear isoform of IL-33 was detected, specifically in epicardial lymphatics, suggesting a role as a transcriptional regulator. Maf, a transcription factor, was also upregulated and prioritized for further study.

Functional studies in human dermal lymphatic endothelial cells showed that overexpression of Il33 and Maf reduced proliferation and increased apoptosis. Transcriptomic analysis revealed downregulation of pathways involved in cytoskeleton organization, extracellular matrix structure, and cell migration. Furthermore, stimulation with age-associated cytokines identified IFN-γ as an upstream regulator of Il33 and Maf.

In summary, aging-associated IFN-γ may drive Il33 and Maf expression, contributing to lymphatic regression. Whether this impairs lymphatic drainage and promotes cardiac pathology is under investigation.

A3-12: TBX4 regulates pulmonary pericyte function in vascular development and pediatric pulmonary arterial hypertension

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The pulmonary vasculature relies on coordinated interactions between endothelial and perivascular cells, but the contribution of pericytes to pulmonary vascular development and disease is not well defined. We identified a previously uncharacterized population of arteriole-associated pericyte-like cells in the mouse lung. scRNAseq showed elevated Tbx4 expression in lung pericytes. To examine its function, we depleted Tbx4 in human lung arterial pericytes and observed altered PDGFRβ and αSMA expression, decreased proliferation, increased apoptosis, upregulated inflammatory pathways, and suppressed angiogenesis-associated programs, as revealed by RNAseq and ATACseq. To evaluate the function of Tbx4 in vivo, we conditionally deleted Tbx4 in pericytes in neonatal and adult mice, resulting in reduced distal pulmonary vascular density in both cases. Given the strong link between TBX4 mutations and PAH in children, we created a Tbx4 mutant mouse model relevant to patients. This model also showed impaired pulmonary vascularisation and developed PAH along with symptoms of small patella syndrome. Notably, both Tbx4 deletion and mutation selectively

A3-13: Targeting Plet1-dependent reparative macrophage pathways to promote lung repair in COPD

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Impaired activation of reparative macrophage programs is a central feature of defective lung repair in COPD, yet the underlying mechanisms and their therapeutic potential remain poorly defined. Effective epithelial repair following lung injury depends on the timely acquisition of reparative macrophage states. Plet1 marks a macrophage population associated with epithelial regeneration, however, the upstream signals and regulatory mechanisms controlling its induction remain incompletely understood. Using in vitro models that mimic epithelial injury, we show that Plet1 expression is induced in macrophages in response to tissue damage–associated cues.

While GM-CSF acts as a potent driver of this response, our data indicate that additional signals contribute to Plet1 induction, pointing to a broader regulatory network activated during epithelial injury. Mechanistically, Plet1 induction is accompanied by increased chromatin accessibility at its genomic locus, consistent with active epigenetic remodeling. Integrative analyses further identify candidate transcription factors, including Nrf2/Bach1, Klf9, Fosl2, and Egr2, that are engaged under these conditions and likely contribute to the establishment of a reparative macrophage state. Extending these findings toward disease relevance, we observe that this regenerative program is impaired in COPD, where macrophages fail to adequately acquire Plet1-associated states. In line with this, therapeutic administration of recombinant Plet1 promotes epithelial repair and improves lung function in preclinical models, supporting the concept that this pathway remains targetable despite chronic injury. Together, our work defines a multi-signal–dependent pathway controlling Plet1 induction and highlights its potential as an entry point to restore macrophage-driven lung regeneration in chronic lung disease.

A3-14: Divergent IL-11 and IL-6 signalling shape cardiac injury outcomes in regenerative vs. non-regenerative vertebrates

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In most mammals, cardiac injury typically results in permanent fibrotic scarring. In contrast, zebrafish exhibit complete, scar-free regeneration of damaged cardiac tissue. Interleukin 11 (IL-11) has previously been identified as a critical mediator of the regenerative response in zebrafish, whereas in mammals it is commonly associated with pro-fibrotic signalling. However, the dynamics of IL-11 expression in early and late phases of the injury response in mammals remains poorly understood. To investigate this, we performed left anterior descending (LAD) artery ligation in wild-type C57BL/6 mice and collected heart tissue for bulk RNA sequencing at 6, 24, and 96 hours post-injury. In parallel, wild-type zebrafish hearts were subjected to cryoinjury, followed by RNA sequencing at 24 and 96 hours. Interestingly, IL-11 was upregulated in both mice and zebrafish at 24 hours post-injury. However, the IL-11 receptor (Il11ra1) was significantly downregulated only in mice. Notably, IL-6 expression and signalling was strongly upregulated at all time points in mice, but not in zebrafish. By 96 hours, fibrotic pathways were activated in mice but were not upregulated in zebrafish. Overall, our data show that although IL-11 is upregulated in both species following cardiac injury, the downstream signalling and outcomes are fibrosis and regeneration in mice and zebrafish respectively. Furthermore, proteomics analysis revealed that IL-11 alone did not significantly upregulate fibrotic protein expression after 24 hours of treatment in mouse epicardial fibroblasts. However, Il11ra1-/- cells displayed reduced expression of fibrotic proteins and downstream signalling. This highlights a dichotomy in IL11ra1-mediated signalling in regenerative versus non-regenerative vertebrates. In particular, IL-6 as well as altering IL11Ra1 signalling in the mouse injury response, represent promising therapeutic targets to promote regenerative repair in the mammalian heart.

A3-15: Restraining inflammation to enable regeneration: Junb as a key Il11-Stat3 effector in the zebrafish (Danio rerio) tissue regeneration model

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With few exceptions, the predominant mode of tissue repair in mammals is scarring due to limited regenerative capabilities. Consequently, injuries result in reduced organ function and are a major contributor to severity and morbidity in human diseases. While low regenerative capacity is widespread among mammals, it is not a general trait of vertebrates. The zebrafish (Danio rerio) is known for high regenerative capacity, where damage to organs or extremities leads to complete regeneration and refunctionalization of the damaged tissue.

Interleukin-11 (Il-11) signaling with the downstream target Junb was identified as a key regulatory pathway in zebrafish regeneration. Junb functions as part of the activator protein 1 complex (AP-1) by dimerizing with proteins from the Fos, ATF or Maf family to bind DNA and regulate transcription. Here we show that injury in the zebrafish leads to increased expression of junba and junbb. This increase is absent in non-regenerative fish lacking the Interleukin 11 receptor (il11ra-/-) revealing Junba/b as targets of the canonical Il-11-stat3 signalling axis.Using junba and junbb mutant alleles, we investigate the role of Junb utilizing the zebrafish larval fin fold regeneration model. Employing genetic models and pharmacological interventions, we prove its importance for regenerative processes in zebrafish through regulation of post injury inflammation.

In junb-/- larvae, additional to impaired regeneneration, tissue damage also leads to severly increased inflammatory markers when compared to regenerative wildtypes. Intriguingly high inflammation is also visible in mammals in the mouse myocardial infarction model, suggesting a connection between high inflammation and the absent ability to regenerate tissue.

Notably, pharmacological intervention with anti-inflammatory drugs restored regeneration in junb-/- confirming Hyperinflammation as the cause for impaired regeneration in Junb deficient zebrafish larvae.

A3-16: Transcriptional regenerative reprogramming leads to the activation of cardiac stromal cells and blastema like feature post cardiac cryo-injury in zebrafish.

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The zebrafish heart possesses the remarkable capacity for scar-free regeneration following tissue loss or damage, providing critical insights into how to combat fibrosis and scarring, a leading cause of heart failure in mammals. To delineate the transcriptional underpinnings of this process, we established a high-resolution single-cell RNA sequencing atlas of cardiac stromal cells in wild-type (WT) zebrafish at different time-points post cryoinjury. Our analysis reveals a rapid dedifferentiation of epicardial and epicardium derived cells (EPDCs) and endothelial cells (EndoCs) within 24 hours in response to cryoinjury. This process is characterized by the transcriptional silencing of mature marker genes such as tcf21, mfap5, or kdrl, fgl2a for EPDCs and EndoCs respectively. Concurrently, the dedifferentiating cells now activate a transient pro-regenerative gene program, including familiar members of the cardiac regenerative gene program such as fn1b or aldh1a2 or rspo3, crlf1a, and others. Together, these processes can be best described as regenerative reprogramming. Notably, processes like regenerative reprogramming as we observed in the heart have been described in other contexts of scar free regeneration such as limb- or fin regeneration where connective tissue cells form a blastema. Here, mapping transcriptional changes during blastema development on our single cell atlas, we show that cardiac stromal cells indeed obtain blastema like transcriptional characteristics. Moreover, utilizing the il11ra mutant which we have previously shown as incompetent for scar free regeneration in fin and heart tissues, we show that Interleukin-11 (IL-11) signaling is essential for regenerative reprogramming. Our analysis uncovers a profound divergence in fate. In the absence of IL-11 signaling, cardiac stromal cells fail majorly to initiate regenerative reprogramming as observed in WT. Instead, EPDCs and EndoCs directly commit to a profibrotic trajectory as evident from elevated e

A3-17: Airway-to-Alveolar Repair Mechanisms during Chronic Cigarette Smoke-Induced Lung Injury

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Lung structural integrity is compromised by chronic environmental exposure to noxious agents like cigarette smoke (CS), leading to COPD development. The loss of developmental signalling in epithelial progenitors impairs repair, ultimately driving emphysema. Fibroblast Growth Factor (FGF) Receptor 2b (FGFR2b) on epithelial cells, including club cell secretory protein (CCSP) positive airway progenitors, is critical for FGF10 signalling and could contribute to alveolar homeostasis.This study investigates lung resilience mechanisms, focusing on how proximal CCSP+ progenitors support distal alveolar repair via FGFR2b signalling during CS injury. We utilised a transgenic mouse model (CCSP_rtTA;Tet(O)_Cre;Fgfr2bflox/floxtdTomato) to target airway/bronchioalveolar stem cells in the lungs of experimental animals. Wild type (WT) and FGFR2b-deficient mice were exposed to CS for three months. Repair was assessed by CCSP+ lineage-labelled cell tracing, while emphysema severity was evaluated by in vivo lung function tests and histological alveolar morphometry.During the CS injury resilience phase, airway CCSP+ progenitors demonstrated regenerative capacity. In exposed WT mice, lineage-labelled CCSP cells successfully contributed to the alveolar type 2 (AT2) cell pool in the distal parenchyma. Deletion of FGFR2b in CCSP+ progenitors abolished this phenomenon, demonstrating that FGFR2b acts as a crucial receptor on CCSP+ progenitors, mediating the FGF10 signals required for alveolar epithelial differentiation. However, at this early CS exposure time-point, WT mice exhibited comparable emphysema severity to mice lacking FGFR2b in CCSP+ progenitors. Extended CS exposure time-points are therefore required to determine the long-term structural consequences of this impaired early reparative capacity. Targeting downstream effectors of the FGF10/FGFR2b axis offers a promising pharmacological strategy to mimic its regenerative potential and drive alveolar repair in compromised lungs.

A3-18: Early-life oxidative stress reprograms an AT2-macrophage signaling circuit causing genomic stress and premature lung aging

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Rationale: Preterm infants with oxygen supplementation are at risk for bronchopulmonary dysplasia (BPD), characterized by structural changes similar to aging lungs. These findings are linked to an increase of M1-like macrophages in the lung. We now investigate the role of early oxidative stress on the macrophage-AT2 interplay promoting premature alveolar aging.

Methods: Neonatal mice were exposed to hyperoxia (HYX, 85% O2), followed by RNA-seq of AT2 cells. IL-6 was knocked out; IL-6 cis/trans and STAT3 pathways were pharmacologically inhibited. Human macrophages and alveolar epithelial cells (hAEC) were studied in vitro. Single nuclei (sn)RNA-seq of BPD lungs and Human Lung Cell Atlas were interrogated.

Results: In neonatal mice, transcriptomic profiling identified a pro-inflammatory, chemoattractant AT2 cell state that promotes M1-like macrophages recruitment to the lung. This AT2-macrophage axis caused a progressive AT2 depletion, with increased

DNA damage response, R-loops formation, single-stranded DNA and RNA synthesis stalling. Il6 knockout, selective pharmacological blockade of IL-6 trans-signaling or STAT3 preserved alveolarization and protected from aging processes. In hAEC, HYX induced IL-6 and genomic instability, promoting macrophage migration. Conversely, HYX-conditioned M1-macrophage secretome caused genomic stress of hAEC. This interplay was abolished by IL-6 trans-signaling inhibition. Finally, snRNA-seq of human BPD lungs confirmed inflammatory and chemoattractant AT2 cells. AT2 Pathways regulated in actively evolving BPD converged with physiological aging. Complementary, AT2 genomic stress was confirmed in BPD lungs.

Conclusion: Our data show that oxidative stress early in life causes an inflamed, genomically stressed alveolar epithelium driving macrophage recruitment and inflammation via IL-6 trans-STAT3 signaling, a pharmacologically promising therapeutic strategy to prevent premature alveolar inflamm-aging in infants at risk for BPD.

AREA 4

A4-1: Spatial and mechanical rewiring of tumor ecosystems in fibrosis-associated lung cancer

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The link between lung cancer and pulmonary fibrosis is associated with a poorer prognosis and a lower survival rate. Cellular plasticity plays a key role in both diseases. It is determined by complex interactions within the tumor microenvironment (TME) that shape its composition and function. However, the spatial architecture of TME components and cell fate plasticity in lung cancer with fibrosis (LCPF) remain poorly understood. This study aims to elucidate the TME landscape and cell-cell communication through spatial profiling, focusing on the identification of unique subpopulations in LCPF and their impact on disease progression. To achieve this, spatial transcriptomics and proteomics were performed on tissue microarray from 18 patients per group in three cohorts: LC, PF and LCPF. Spatial proteomics analysis revealed that more proliferating and fewer apoptotic tumor cells are present in LCPF, accompanied by a distinct spatial distribution of immune cells. In particular, the number of immune cells, especially macrophages, was reduced in LCPF compared to LC. The higher proportion of proliferating tumor cells in combination with suppressed immune cell populations reflects the increased invasive potential of tumor cells in LCPF. Spatial gene profiling identified a unique tumor cell subpopulation in LCPF characterized by high metabolic gene expression. Comparative analysis of LC and tumor regions in LCPF revealed activation of mechanotransduction and fatty acid metabolism in the tumor region. In parallel, serum proteomics from 30 LC and LCPF patients showed increased cell adhesion pathways in LCPF. In conclusion, our study reveals cellular subpopulations and potential pathways for improved therapies in fibrosis associated lung cancer.

A4-2: Orphan GPCR dimerization in macrophages: physiological relevance and pharmacological modulation

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Macrophages express numerous G protein-coupled receptors (GPCRs) that regulate adhesion, migration, and activation, but the function of orphan receptor GPRC5B in macrophages is unknown. Both resident peritoneal and bone marrow-derived macrophages from myeloid-specific GPRC5B-deficient mice show increased migration and phagocytosis, resulting in improved bacterial clearance in a peritonitis model. In other models such as myocardial infarction, increased myeloid cell recruitment has adverse effects. Mechanistically, we found that GPRC5B physically interacts with GPCRs of the prostanoid receptor family, resulting in enhanced signaling through the prostaglandin E receptor 2 (EP2). In GPRC5B-deficient macrophages, EP2-mediated anti-inflammatory effects are diminished, resulting in hyperactivity. Using in silico modelling and docking, we identify residues potentially mediating GPRC5B/EP2 dimerization and show that their mutation results in loss of GPRC5B-mediated facilitation of EP2 signaling. Finally, we demonstrate that decoy peptides mimicking the interaction interface and small molecules disrupting this interaction reduce GPRC5B-mediated facilitation of EP2-induced cAMP signaling in macrophages

A4-3: AKR1A1 is a denitrosase in endothelial cells and impact on endothelial cell metabolism

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Background: Aldo-keto reductase family 1 member A1 (AKR1A1) is traditionally recognized for its role in biogenic aldehyde reduction. However, recent evidence suggests it also functions as a denitrosylase, regulating protein S-nitrosylation. Therefore, the goal of this study was to assess whether it affects nitric oxide (NO) signalling and function in endothelial cells. Methods: AKR1A1 levels were modulated via siRNA-mediated knockdown and overexpression approaches. We employed a multi-layered analysis integrating transcriptomic profiling (RNA-seq), proteomic and untargeted metabolomics. Protein S-nitrosation was assessed using the biotin switch technique (BST). Functional validation included fibronectin adhesion, monocyte-endothelial interaction, Proliferation and spheroid-based sprouting assays. Results: AKR1A1 was expressed by cultured endothelial cells under basal conditions and its siRNA-mediated knockdown resulted in altered endothelial cell metabolism. LC-MS/MS based metabolomics analyses revealed that the lack of AKR1A1 increases metabolites that are linked to NO signaling. These included L-citrulline, the nucleotides AMP and GMP the pentose phosphate pathway intermediate; ribose 5-P as well as glutathione species (GSH and GSSG). The latter changes could be accounted for by the S-nitrosation of pyruvate kinase M2 (PKM2). Indeed, it was possible to demonstrate using the BST assay that the overexpression of AKR1A1 decreased the S-nitrosation of PKM2. Transcriptomic and Proteomic analysis reveled that AKR1A1 may potentially affect pathways linked to NO. This was confirmed in as much as eNOS expression increase in AKR1A1 depleted cells, at the same time ICAM-1 and VCAM-1 expression was reduced, as was monocyte adhesion. There were also significant changes in genes linked with G protein coupled receptor signalling and extracellular matrix regulation. Fitting with these observations AKR1A1-deficient cells adhered better to fibronectin and demonstrated a pro-angiogenic phenotype i.e. both migration and sprouting from endothelial cell spheroid were increased. Conclusion: Our data highlight the importance of AKR1A1 as a critical denitrosase in the endothelium that regulates PKM2 S-nitrosylation and endothelial cell metabolism as well as function. These results suggest AKR1A1 may be a target in strategies to improve endothelial cell function and re-establish the S-nitrosoproteome in conditions of low NO bioavailability.

A4-4: CASC15 as a Regulator of Endothelial Function in Vascular Aging and Disease

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Endothelial dysfunction and senescence are central drivers of vascular aging and diseases such as atherosclerosis and abdominal aortic aneurysm (AAA). Long non-coding RNAs (lncRNAs) have emerged as critical regulators of endothelial homeostasis, yet their roles in senescence-associated vascular remodeling remain poorly defined. Here, we aimed to identify lncRNAs with potential regulatory functions in endothelial aging and disease.

CASC15 was among the most strongly deregulated lncRNAs in senescent HUVECs (Log2FC: 1.96; padj<0,001) and displayed altered expression in single-cell RNA sequencing datasets from AAA (Log2FC −4.316; p = 0.368) and atherosclerotic tissues (Log2FC 1.624; p = 0.96) when comparing advanced lesions to adjacent early lesions. Functional studies using gapmers to downregulate CASC15 in HUVECs revealed significant reductions in proliferation (≈50%, p = 0.027) and migration (≈40%, p < 0.0001), along with increased expression of inflammatory markers under low inflammatory stimulation ($0^{,5}$ ng/ml TNF-α). Knockdown of CASC15 also impaired angiogenesis under basal conditions, an effect rescued by VEGF-A stimulation. CASC15 overexpression enhanced basal sprouting without further effect of VEGF-A stimulation. Mechanistically, CASC15 regulates nearby SOX4 expression, which is partly responsible for the phenotype after CASC15 knockdown. Additionally, ATAC-seq profiling following CASC15 knockdown revealed widespread changes in chromatin accessibility, suggesting that CASC15 may further regulate endothelial gene expression through modulation of the chromatin landscape.

These findings indicate that CASC15 contributes to endothelial homeostasis, angiogenic potential, and inflammatory responsiveness, suggesting a role in vascular remodeling processes relevant to AAA and atherosclerosis.

A4-5: Rnase T2 as a macrophage-associated inflammatory regulator in HFpEF

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Background

The incidence of heart failure with preserved ejection fraction (HFpEF) is increasing concomitantly withpopulation ageing and the growing prevalence of metabolic disorders. Current therapeutic guidelines recommend use of sodium-glucose cotransporter-2 inhibitors of glucagon-like petide-1 agonists, which target metabolic risk but do not directly address pathological mechanisms within the myocardium. Consequently, a substantial biological mandate remains for the discovery of cardiac-specific therapeutic targets for the treatment of HFpEF.

Methods

We applied a novel algorithm based on explainable ar5ficial intelligence and deep neural networks to identify genes discriminating distinct heart failure phenotypes at a single-cell level. Candidate genes were validated using an independent bulk RNA-sequencing dataset from pa5ents with HFpEF. Functional relevance was assessed in vitro using THP-1-derived macrophages and subjected to siRNA-mediated gene silencing.

Results

Differentiation gene contribution analysis identified 12 candidate genes distinguishing HFpEF cells. Among these, RNase T2 was consistently ranked as an important contributor and was significantly upregulated in HFpEF. Expression of RNase T2 was increased in both resident LYVE1+ and recruited LYVE1- macrophages in HFpEF patients. These findings were independently validated in a bulk sequencing cohort and corroborated by elevated plasma RNase T2 levels measured by ELISA in patients with HFpEF. Func5onal interroga5on in macrophages demonstrated that RNase T2 silencing selec5vely reduced IL-6 secre5on in response to single-stranded RNA40 (ssRNA40) s5mula5on, while baseline inflammatory signalling and macrophage polarisa5on remained unaffected.

Conclusion

RNase T2 is implicated in HFpEF and may regulate macrophage inflammatory response. Further mechanistic insights are required to define the relevance of RNA-sensing recogni5on and signalling in the context of HFpEF.

A4-6: Pericyte contact alters endothelial cell metabolism by promoting exchange of lactate through SLC16A3

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Dynamic communication between endothelial cells and pericytes is essential for vascular development and stability; however, the metabolic basis of this interaction remains insufficiently understood. To address this, we employed a filter-based co-culture system that recapitulates the shared basement membrane of the microvasculature and performed integrated multi-omics analyses to determine how direct cell–cell contact influences both cell types. We observed that initial contact did not immediately induce endothelial quiescence but instead triggered a transient activation of an endothelial-to-mesenchymal transition (EndMT)-like program. This response involved selective upregulation of extracellular matrix-associated genes, including collagen isoforms and PDGFR signaling components, while overall endothelial identity remained preserved.

Concurrently, pericytes reprogrammed endothelial metabolism by enhancing glycolysis and elevating intracellular pyruvate and lactate levels. Proteomic profiling of the contact interface revealed enrichment of solute carrier proteins, most prominently the lactate transporter SLC16A3. Functional experiments demonstrated that pericytes act as a glycolytic partner, transferring lactate to endothelial cells via SLC16A3. Rather than fueling the tricarboxylic acid cycle, lactate functioned as a signaling metabolite, promoting widespread remodeling of the endothelial acetylome and lactylome, particularly affecting glycolysis-related proteins.

In vivo validation showed that endothelial-specific deletion of SLC16A3 impaired postnatal retinal angiogenesis, reducing vascular density and endothelial–pericyte association without altering endothelial proliferation, indicating metabolically regulated cellular plasticity coordinates vessel maturation.

A4-7: Endothelial cytochrome P450 reductase-derived cholesterol limits angiogenesis

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The cytochrome P450 reductase (POR)/CYP51 monooxygenase is a redox system important for sterol synthesis. Cellular cholesterol is determined by uptake and de novo synthesis. High cholesterol is linked to cardiovascular disease, but the role of endogenous cholesterol synthesis in endothelial function, in contrast, is unknown. To inhibit cholesterol synthesis in endothelial cells, POR and CYP51 CRISPR knockout was performed in human aortic endothelial cells (HAEC) and HUVEC. Furthermore, an endothelial-specific tamoxifen-inducible POR knockout mouse (ecPOR-/-) was generated. Knockout of POR and CYP51 in HAEC led to accumulation of the CYP51 substrate lanosterol, whereas its product desmosterol was reduced. Functionally, loss of endogenous cholesterol synthesis was linked to increased angiogenic sprouting in HUVEC. Similarly, endothelial sprouting from aorta was increased in ecPOR-/- mice compared to control mice. Importantly, increased angiogenesis was also observed in vivo in the retina of ecPOR-/- mice. Cellular cholesterol levels are sensed by SREBP2 (sterol regulatory element-binding protein 2), and indeed its activation (cleavage and nuclear translocation) was increased after deletion of POR in cultured cells as well as in vivo (en face aorta). Overexpression of active, cleaved nuclear SREBP2 increased angiogenesis similar to POR knockout. RNA-seq of POR-/- HAEC showed significant upregulation of cholesterol-related genes LRP1, SC5D, and HMGCS1 as well as those of the angiogenic PI3K/AKT-pathway such as ANGPT2 and JAK3. qPCR analysis after SREBP2 and POR/SREBP2 double knockout showed that induction of both cholesterol and angiogenesis pathways is SREBP2-dependent and suppressed in the double knockout, as demonstrated by genes such as HMGCS1 and ANGPT2. Together, these findings show that inhibition of endothelial cholesterol synthesis activates SREBP2 to drive pro-angiogenic gene expression, identifying the POR/CYP51–SREBP2 axis as a key regulator of angiogenesis.

A4-8: A microprotein encoded by FERMT3 modulates endothelial cell protein catabolism and induces cell cycle arrest and senescence

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We identified 2,244 previously uncharacterized human endothelial microproteins (miPs) encoded by non-canonical small open reading frames (smORFs). In this study, we characterized a novel 69-amino-acid microprotein derived from the FERM domain-containing kindlin-3 transcript (miP-FERMT3), which is upregulated under inflammatory conditions. In endothelial cells, miP-FERMT3 localizes predominantly to centriole subdistal appendages, where it colocalizes with ninein and CEP170 and drives centrosome amplification. Its expression induces cell cycle arrest and DNA damage, as evidenced by γ-H2AX foci formation and nuclear p53 accumulation. Transcriptomic analysis revealed downregulation of genes involved in cell-cycle progression and upregulation of cell cycle inhibitory and senescence-associated programs. Notably, cell cycle arrest occurred independently of canonical p53 target gene activation. Mechanistically, miP-FERMT3 interacts with components of the ubiquitin–proteasome system including PSMD9, CUL2 and TRIM8 and enhances global protein ubiquitination, centrosomal neddylation and proteasome activity. This increased proteasomal activity promotes degradation of p21, leading to replication stress, as indicated by elevated CHK1 phosphorylation. Consequently, miP-FERMT3 induces a rapid senescence phenotype characterized by increased cell size, β-galactosidase activity, telomere shortening, DNA damage, loss of proteastasis and the induction of a paracrine pro-inflammatory response in naïve endothelial cells. Importantly, analysis of independent murine and human aging datasets revealed increased FERMT3 expression and protein abundance with age. Together, our findings identify miP-FERMT3 as a novel regulator of endothelial cell fate that links centrosomal protein homeostasis and ubiquitin-proteasome activity to cell cycle control and vascular senescence, suggesting a potential role in age-associated vascular dysfunction.

A4-9: Acute myocardial infarction induces long-term epigenetic remodeling in endothelial cells

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Myocardial infarction (MI) induces endothelial cell (EC) death, inflammation, and transient mesenchymal transition. Here, we show that acute myocardial infarction (AMI) causes long-term alterations in chromatin accessibility in ECs, particularly at loci regulating endothelial antigen presentation and senescence.

To investigate persistent epigenetic changes after MI, we performed single-nuclei ATAC-sequencing on mouse hearts 28 days post-infarction. We observed increased chromatin accessibility at the Ciita locus, a key regulator of major histocompatibility complex class II (MHCII) expression and T-cell activation. Consistently, Ciita expression was elevated in ECs from infarcted mouse hearts and in single-nuclei RNA-sequencing data from patients with ischemic heart failure. Ciita expression and spatial localization were further validated using advanced spatial transcriptomic approaches and RNAscope in mouse heart sections, and confirmed in human spatial transcriptomics datasets.

Functionally, AAV-mediated endothelial-specific knockdown of Ciita after MI perserved cardiac function and reduced T-cell accumulation in the infarct region. Together, these findings highlight a critical role for sustained endothelial–immune cell interactions following cardiac injury and identify endothelial CIITA as a potential therapeutic target to preserve cardiac function after MI.

A4-10: Elastogenesis in the pregnant uterus as a paradigm to reinstate elasticity in organs

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Introduction:

Elastolysis, the loss of elastic fibers, contributes to severe diseases like aortic aneurysms. The uterus is the only known adult mammalian tissue capable of reactivating elastogenesis, producing elastic fibers during pregnancy-induced expansion. Understanding the molecular mechanism governing this process could permit the development of therapeutic approaches to tackle elastolytic diseases.

Methods:

Time-resolved single-nuclear RNA-sequencing was used to analyze elastogenesis related gene regulatory networks in the pregnant uterus and compare them to cardiac tissue. Isolated fibroblasts were stimulated with pregnancy hormones to explore mechanisms driving elastic gene expression.

Results:

Elastogenesis-associated genes (Eln, Fbn1, Fbn2) show a rapid increase in uterine tissue during pregnancy. At single-nuclear level, cell type-specific expression patterns identify uterine fibroblasts and SMC's as key players, with both cell types upregulating Eln and Fbn1. Time-resolved gene regulatory network analysis in the heart and uterus suggests that unique transcription factor cascades terminating in elastogenesis are present in the uterus, which may be controlled by Foxo1 and Esrrg. In vitro experiments with isolated fibroblasts demonstrated that estrogen and progesterone provoke antagonistic effects on elastogenesis-related gene expression, with estrogen upregulating of Eln and Fbn1. These results hint that hormonal changes upon pregnancy may stimulate elastogenesis in a tissue-specific manner, as determined by receptor expression.

Conclusion:

In conclusion, elastogenesis in the pregnant uterus is driven by hormonal activation of gene regulatory networks targeting key elastogenic genes. While this network is dormant in the adult cardiovascular system, identifying critical molecular junctions could reactivate elastogenesis, offering novel therapeutic avenues for diseases like aortic aneurysms.

A4-11: Substrate stiffness induces senescence via the secretion of YBX1 by endothelial cells

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Cardiac ageing is marked by fibrosis, vascular dysfunction, inflammation, and senescence-associated signalling. Fibrosis increases myocardial stiffness, but how endothelial cells respond to the stiffening remains unclear.

To address this question, we have cultured endothelial cells (EC) on matrices to mimic healthy (8 kPa) and fibrotic (63 kPa) myocardium using Sylgard 184, a non-reactive polymer. ECs cultured on stiff matrices resembled aged cardiac ECs. Specifically, they presented increased size, shorter telomeres, signs of senescence, increased expression of ACTA2 and cytokines like IL1B and IL6 and reduced endothelial cell adhesion.

EC regulate their microenvironment via secretion of angiocrine signals. Treating naïve EC with supernatant of EC cultured on stiff matrices, impaired barrier, increased cell size, reduced nutrient uptake, and increased senescence. Our data suggests that increased stiffening is an important trigger for the acquisition of ageing-related hallmarks in EC. Proteomic profiling of EC supernatant identified 102 proteins enriched on stiff matrices. Y-box binding protein 1 (YBX1), a stress-induced extracellular mitogen, was enriched in stiff supernatant. Treating EC with YBX1 reduced nutrient uptake, increased β-galactosidase activity, senescence related gene expression and NADPH-dependent dehydrogenase activity. These findings suggest that stiffness induced secretion of YBX1 may induce endothelial senescence. Interestingly, spatial transcriptomics analysis of young and old murine hearts revealed that the enriched expression of YBX1 in senescence hotspots in the heart. YBX1 has been reported to be a ligand of Notch4. DAPT treatment rescued YBX1 senescence induction suggesting that in EC YBX1 induces via Notch activation.

In conclusion, our findings suggest that myocardial stiffness induces age-like effects in endothelial cells. EC secrete stiffness associated secretome that induces cellular senescence in a YBX1-Notch dependent mechanism.

A4-12: THAP1-dependent chromatin export by macrophages shapes the lung tumor microenvironment

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Disruption of tissue integrity and increased microenvironmental permeability are defining features of lung cancer, shaping tumor progression, immune cell trafficking and inflammatory signaling. Tumor-associated macrophages (TAMs) occupy specialized niches within the tumor microenvironment (TME) and coordinate local remodeling, yet prevailing models largely attribute their effects to soluble mediators. Whether macrophage nuclear programs generate structured extracellular signals remains unclear. Here we identify regulated chromatin export by TAMs as a distinct signaling modality. Although extracellular chromatin (nucleosomes and histones) is typically viewed as a byproduct of cell death or extracellular trap formation, emerging evidence—including extranuclear histone pools, extracellular vesicle-associated histone trafficking with distinct post-translational modifications, and vesicular DNA export—suggests that extracellular chromatin burden may be actively controlled. Integrated RNA-seq and ATAC-seq of human lung TAMs reveal differential chromatin accessibility with enrichment of THAP1 motifs. Re-analysis of lung adenocarcinoma single-cell datasets predicts communication between THAP1$^+$ TAMs and TME compartments, with enrichment of integrin-containing complexes consistent with adhesion-dependent signaling niches. Mechanistically, THAP1 regulates H2B cluster transcription and abundance, and its knockdown reduces extranuclear H2B and extracellular DNA without affecting proliferation or viability, indicating a non-lytic export pathway independent of extracellular trap or inflammasome programs. Functionally, depletion of THAP1 or H2B reduces extracellular nucleosome-associated material, and conditioned media from control TAMs perturbs barrier properties in a DNase- and protease-sensitive manner. Together, these findings define an epigenome-to-extracellular chromatin axis through which macrophages regulate extracellular chromatin burden to shape TME stability, revealing a

A4-13: The effect of mitoTEMPO on the development of hypoxia-induced pulmonary hypertension in male mice

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Mitochondrial reactive oxygen species (mtROS) have been implicated in the development of chronic hypoxia-induced pulmonary hypertension (PH), potentially through hypoxia-inducible factor-1α (HIF-1α) stabilization. However, the role of mtROS in HIF-1α stabilization, PH development and the therapeutic potential of antioxidant treatment remains controversial. Mice were exposed to hypoxia (10% O2) for four weeks to induce PH and treated with mitochondria-targeted antioxidant mitoTEMPO or its carrier control, triphenylphosphonium (TPP+). Cell proliferation was evaluated in mouse pulmonary arterial smooth muscle cells (PASMCs) by BrdU incorporation. HIF-1α stabilization and downstream target expression were investigated in mouse lung carcinoma epithelial (CMT167) cells and isolated mouse and human PASMCs under different hypoxic conditions. In vivo mitoTEMPO treatment did not affect chronic hypoxia-induced PH compared to TPP+. In vitro, mitoTEMPO treatment did not inhibit hypoxia-induced mouse PASMCs proliferation, but enhanced proliferation under normoxic conditions.

In vitro exposure of CMT167 cells and mouse or human PASMCs to mitoTEMPO or TPP^+ did not alter HIF-1α protein levels or expression of its downstream targets lactate dehydrogenase A (Ldha) and pyruvate dehydrogenase kinase 1 (Pdk1) under normoxic (21% O_2) or hypoxic (1% or 10% O_2) conditions after 24h.

These findings do not support a therapeutic benefit of mitoTEMPO in hypoxia-induced PH.

A4-14: From Infiltration to Identity: Macrophage Reprogramming Fuels Pulmonary Vascular Remodeling in COPD-associated Pulmonary hypertension

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Patients with chronic obstructive pulmonary disease (COPD) frequently develop pulmonary hypertension (PH) and cor pulmonale. Even mild PH affects patient survival. Intriguingly, pulmonary vascular alterations occurs prior to emphysema development. Our previous studies demonstrated the critical role of macrophages in driving pulmonary vascular remodeling in COPD-PH. However, events controlling monocyte recruitment and macrophage phenotypes in this pathogenic cascade remain poorly understood.

Our study revealed that recruitment of monocyte-derived macrophages and proliferation of pulmonary vascular cells increased after 1 month of cigarette smoke (CS) exposure in mice. Transcriptomic analysis identified a distinct CS-induced macrophage subpopulation characterized by differential gene expression patterns linked to cell proliferation and extracellular matrix remodeling. To decipher the mechanism of monocyte recruitment upon CS exposure, we performed chemokine screening and detected upregulation of chemokine (C-C motif) ligand 3 (Ccl3) at early (1 month) and later (3 and 8 months) time points. Neutralization of Ccl3 and its receptors reduced the chemotaxis of monocytes toward bronchoalveolar lavage fluid from CS-exposed lungs.

Altogether, these findings suggest that CS-exposure can trigger Ccl3-mediated macrophage infiltration into the lung, and change their transcriptomic profile, which collectively may initiate pulmonary vascular remodeling and diseases progression.

A4-15: Atherosclerosis impairs lung repair and alveolar macrophage function following SARS-CoV2 infection in LDLr$^{-/-}$ mice.

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Cardiovascular diseases (CVDs) and atherosclerosis are strong predictors of adverse COVID-19 outcomes, yet the mechanisms linking atherosclerosis to impaired recovery remain unclear. To address this, we analyzed SARS-CoV-2–induced pulmonary responses in LDLr$^{-/-}$ mice fed a fat-rich diet to induce atherosclerosis. Although viral RNA levels and survival were comparable between groups, infected LDLr$^{-/-}$ mice showed elevated BALF protein and LDL activity, indicating persistent alveolar–capillary barrier dysfunction. Flow cytometry demonstrated sustained elevation of CD45^{+} immune cells in BALF, predominantly bone-marrow–derived macrophages (BMDM) and CD11b+-tissue-resident alveolar macrophages (TR-AM). scRNA-seq revealed the emergence of a specific alveolar TR-AM subset characterized by upregulation of antigen-presentation, lysosomal, and stress-response pathways, together with downregulation of phagocytic, cytoskeletal, and inflammatory modules in lungs of atherosclerotic mice. In addition, tissue resident macrophages displayed stress-adaptive and immunoregulatory signatures rather than homeostatic repair phenotypes. In contrast to WT mice, which restored lung epithelial compartments (AT1, AT2, ciliated, goblet, and bronchioalveolar stem cells [BASCs]) by 28 dpi, LDLr$^{-/-}$ lungs showed persistent depletion of BASCs, goblet, and multiciliated cells. These results demonstrate that dyslipidemia leads to specific macrophage reprogramming in COVID-19, maintain immune-cell infiltration, and impair epithelial regeneration independently of viral replication. These findings provide mechanistic insight into the heightened susceptibility of individuals with atherosclerosis to severe and prolonged COVID-19 lung pathology.

A4-16: Perinatal Obesity Promotes Cardiac Aging and HFpEF Phenotypes via WAT-Heart communication and Early Activation of DNA Damage Response

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Background: Maternal and early-life obesity can increase heart disease risk through metabolically driven inflammation. Since meta-flammation and aging converge in similar pathways, we investigated if perinatal obesity causes cardiac remodeling by prematurely activating hallmarks of aging in the offspring.

Methods: Offspring of high-fat diet (HFD)- or standard-diet (SD)-fed dams were analyzed at postnatal day 21 (P21), P70, after 1 and 1.5 years, including ventricle-specific transcriptomics. Proteomics were performed on white adipose tissue (WAT) at P21. Precision-cut heart slices (PCHS) were treated with conditioned media of WAT of HFD-offspring (CMWAT-HFD).

Results: Perinatal obesity caused ventricular remodeling with cardiomyocytes hypertrophy and increased collagen deposition. This early cardiac remodeling was linked to functional impairment at P70 that was evident even after 1.5 year. Molecular analyses revealed increased γH2AX, indicating early DNA damage response (DDR) and premature cardiac aging after perinatal obesity. WAT proteomics identified pathways of inflammation in HFD at P21. Integration of WAT-proteomic and heart-RNA-seq data revealed an across tissue communication route. Incubation of PCHS with CMWAT-HFD induced DDR, consistent with a WAT-heart axis. Furthermore, RNA-seq at P21 revealed inflammation- and cardiomyopathies-related signatures and pathways induced by perinatal obesity that resemble those seen in physiological cardiac aging 1 year. Finally, integration of transcriptomic findings at P21 with several murine and rat models of HFpEF exhibited similar expression patterns of genes which are known to be determining HFpEF.

Conclusion: Our data demonstrate that perinatal obesity induces a premature structural and functional cardiac decline, a process that is partly mediated by a WAT-heart axis. The identification of HFpEF pattern early in life opens avenues for therapeutic intervention to prevent cardiac decline later in life.

A4-17: Pharmacological inhibition of STAT3 prevents Notch3-associated vascular remodeling and depletion of vascular pericytes in experimental model of BPD

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Rationale: Bronchopulmonary dysplasia (BPD), a neonatal chronic lung disease, is characterized by vascular hypoplasia and remodeling, leading to higher risk for pulmonary hypertension (PH). Notch3 is a major contributor to pulmonary vascular smooth muscle cells (PVSMC) proliferation. Here, we study the role of Notch3 and STAT3 in vascular hypoplasia and remodeling in experimental BPD.

Method: We employed a hyperoxia-based model of BPD characterized by 85% (HYX) or 21% (NOX) O2. Transcriptomic and proteomic profile of Notch3 was assessed. The STAT3 signaling was targeted by pharmacological blockade using STATTIC. Lung vascular remodeling was assessed with 3D-imaging. Cultured human pulmonary artery smooth muscle cells (HPASMC) were treated with conditioned media of hyperoxia-exposed M1-like macrophages (CMM1-HYX).

Results: First, quantitative histomorphometry showed that HYX reduced microvascular formation, promoted proliferation of PVSMC, and increased vascular muscularization. These structural findings were linked to decreased PDGFRb+ and PDGFRa+ cell populations, assessed by immunofluorescence staining. Second, analysis of scRNA-seq from HYX-exposed neonatal mice revealed differential regulation of Notch3 in pericyte and SMC populations. Complimentary western blot showed, diminished Notch3 gene and protein abundance in total lung homogenates after HYX. Conversely, STAT3 inhibition protected from proliferative PVSMC, vascular remodeling and hypoplasia, preserving PDGFRb and PDGFRa cells after HYX, with a more pronounced effect in PDGFRa- population. Finally, treatment with CMM1-HYX reduced Notch3 expression in HPASMCs.

Conclusion: Our data demonstrate that STAT3 inhibition prevents the proliferation of PVSMC and vascular remodeling, possibly through preservation of Notch3 signaling and pericyte populations. Therefore, targeting STAT3 could offer a potential therapeutic strategy for BPD-associated PH.

A4-18: TMAO - A Missing Metabolic Link Between Environment, Gut Microbiome, and Lung Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is characterized by aberrant tissue repair and persistent myofibroblast activation. As fibroblast metabolism tightly shapes phenotype and matrix-remodeling capacity, metabolomic profiling offers a powerful lens into pathways driving fibrosis.

UPLC-MS/MS analysis of primary lung fibroblasts from 40 explanted lungs (20 IPF, 20 donor) revealed extensive metabolic rewiring across 579 metabolites in 94 pathways, including altered lipid species, dipeptides and glycolysis-related intermediates. We identified 122 upregulated and 5 downregulated metabolites. Pathway enrichment highlighted hexosylceramides, (dihydro-)sphingomyelins and dipeptides, while fgsea indicated changes in acyl-carnitines, monohydroxy-fatty acids and glycolytic intermediates. Random-forest modeling achieved 87.75% accuracy and nominated glycosyl-ceramides, myo-inositol, trimethylamine N-oxide (TMAO), 2'-O-methyluridine, N-acetylmethionine-sulfoxide, sphinganine and N6-acetyllysine as top classifiers. Notably, the gut-microbiota- and air-pollution-derived metabolite TMAO emerged as a strong discriminator, suggesting a previously unrecognized gut-liver-lung axis in IPF. Plasma analyses further indicated elevated TMAO levels in IPF patients.

Functional perturbation experiments showed that TMAO induces replication stress, activates ER-stress pathways, drives metabolic reprogramming and lipid accumulation, and increases collagen I production, collectively reinforcing a profibrotic state. As its precursor trimethylamine (TMA) arises from gut microbial metabolism and airborne sources - and may be amplified through increased FMO3 expression in IPF fibroblasts and tissue - the TMA/TMAO axis likely represents a dual-origin, self-reinforcing metabolic signal relevant to fibrogenesis.

A4-19: Versican dysregulation drives pathological ECM remodeling in pulmonary fibrosis

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A healthy extracellular matrix (ECM) architecture is fundamental to lung function and tissue regeneration, whereas its aberrant remodeling and excessive matrix deposition are hallmark features of idiopathic pulmonary fibrosis (IPF). Versican, a multifunctional proteoglycan and integral ECM component, has been increasingly recognized as a crucial influencer of lung fibrosis. Emerging evidence indicates that versican exerts cell-type-specific and context-dependent functions, acting both as a homeostatic structural scaffold and a pro-inflammatory driver of fibrosis. While publicly available transcriptomic data confirm significant versican upregulation in IPF, downstream mechanisms underlying versican's functional dichotomy remain unclear. We hypothesize that this pleiotropy arises from the synergy between versican core protein isoforms and their glycosaminoglycan (GAG) chain attachments, including differences in chain length and sulfation patterns, which co-regulate the biological responses.

Our analyses of lung homogenates at transcript and protein levels show that versican isoforms V0 and V1, rather than V2 and V3, are increased in IPF compared with donor tissue. This upregulation is recapitulated in primary lung fibroblasts, where IPF-derived fibroblasts exhibit higher V0 and V1 mRNA expression than those from donors. Histological analysis of IPF lung tissue reveals a differential distribution of versican, with low expression in morphologically normal lung parenchyma and markedly increased levels in extensively remodeled fibrotic regions. Finally, native gel electrophoresis revealed altered migration of V0 and V1 in IPF homogenates, suggesting shifts in GAG chain composition and length. Collectively, this ongoing study provides initial insight into how versican isoform and structural heterogeneity contribute to the regulation of ECM homeostasis in IPF.

A4-20: Epiregulin promotes expansion of aberrant airway epithelial cells, driving epithelial-mesenchymal miscommunication in lung fibrosis

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Aberrant mesenchymal-epithelial communication is a central driver of tissue remodeling in pulmonary fibrosis, with Epidermal Growth Factor Receptor (EGFR) signaling acting as a key mediator of this process. Among the seven EGFR ligands, epiregulin (EREG) is unique in its ability to induce sustained receptor activation, resulting in prolonged downstream signaling and maladaptive cellular responses.

Here, we show that EREG levels are elevated in lung homogenates, plasma, and bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis (IPF) compared to donors. Immunolocalization studies revealed EREG expression in aberrant epithelial cells within bronchiolized regions of fibrotic lungs.

Using in vitro and ex vivo systems, including air-liquid interface cultures, bronchiolar organoids, and precision-cut lung slices, we demonstrate that EREG drives the expansion of aberrant secretory-primed basal cells and hillock cells. Transcriptomic profiling of these cell populations identified dysregulation of Wnt signaling, epithelial cell proliferation, regulation of migration, and extracellular matrix organization. Consistently, EREG-primed mouse bronchioalveolar lung organoids (BALOs) triggered expention of mesenchymal cells along with increased expression of extracellular matrix components.

Importantly, inhibition of EREG attenuated bleomycin-induced lung fibrosis in mice, as evidenced by reduced Ashcroft scores, improved lung function, and increased lung volume.

Collectively, these findings identify EREG as a critical regulator of aberrant epithelial transdifferentiation that promotes fibroblast expansion and fibrosis progression, highlighting EREG as a potential therapeutic target in pulmonary fibrosis.

A4-21: Spatial proteomic architecture reveals oncogene-specific immune niches and prognostic signatures in NSCLC

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Only about 20% of NSCLC patients benefit from immunotherapy, partly due to limited understanding of how oncogenic mutations shape the tumor microenvironment (TME). To address this issue, we used 41-marker high-plex multiplex immunofluorescence to profile EGFR and KRAS-mutated tumors and non-mutated NSCLC controls, revealing mutation-specific immune transformations. EGFR- and KRAS-mutated tumors showed higher tumor cell density and lower immune infiltration. Quantitative phenoptic analyses revealed a depletion of cytotoxic T cells, dendritic cells and granulocytes in mutant TMEs, with EGFR-mutated tumors additionally being enriched in M2-like polarized tumor-associated macrophages (TAMs), suggesting an immunosuppressive milieu. We performed three complementary spatial analyses: (i) Cellular neighborhood analysis identified 14 spatial clusters, with reduced cytotoxic and helper T cell–rich neighborhoods in mutant tumors. (ii) Nearest neighbor analysis (nearest neighbors = 5, 10, 20) revealed shorter distances between M2-likeTAMs and proliferating tumor cells in EGFR-mutated tumors and greater immune exclusion in non-mutants. (iii) Spatial proximity analysis (25, [5]0, 100 µm) revealed higher densities of T cells, dendritic cells and macrophages near tumor cells in KRAS-mutated tumors. All spatial metrics correlated significantly with prognosis in Cox proportional hazards models, highlighting immune cell positioning as an important predictor of outcome. These results demonstrate that EGFR and KRAS mutations impact the spatial immune landscape of NSCLC, providing information for mutation-directed immunotherapy strategies.

AREA 5

A5-1: Identification and Characterization of transiently occurring, regenerative MSCs in Influenza Virus-induced Acute Lung Injury

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Influenza A virus (IAV) infection causes severe epithelial damage and high morbidity. Mesenchymal stromal cells (MSCs), positioned between the respiratory epithelium and vasculature, are ideally located to integrate signals during injury and repair. Although MSCs are increasingly recognized for supporting lung regeneration, mechanisms underlying their infection-induced responses remain poorly understood.

Here, we mapped MSC diversity during IAV infection and identified cellular interactions orchestrating injury resolution and regeneration. Using single-cell transcriptomics with spatial and functional assays, we demonstrate pronounced heterogeneity across different lung compartments, including dynamic immune shifts, transient intermediate epithelial cell states, and the emergence of a distinct MSC subset expressing Neuronal regeneration related protein (Nrep). Nrep+ MSCs appear transiently during early recovery stages and localize primarily to damaged regions of the lung. Trajectory analysis indicates that Nrep+ MSCs originate from adventitial fibroblasts and subsequently transition to alveolar fibroblasts. Nrep+ cells are enriched in WNT signaling genes and share a transcriptional signature with non-invasive Sfrp1 fibroblasts. Ligand–receptor analyses identify transitional MSCs as major signaling hubs that interact with multiple epithelial and stromal subsets. Notably, Nrep+ MSCs uniquely secrete growth factors including Midkine (MDK), which promotes lung organoid formation in 3D co-cultures, while MDK inhibition reverses this effect.

In conclusion, we define a novel regenerative MSC population that transiently emerges at early recovery stages and uniquely secretes growth factors to support lung regeneration after IAV infection.

A5-2: Ceramide-induced lipid droplet formation regulates endothelial insulin sensitivity

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Diet-induced obesity promotes insulin resistance and increases the risk of cardiovascular diseases. Already short-term high-fat diet (HFD) feeding has been reported to induce glucose intolerance and insulin resistance; however, the underlying mechanisms and in particular the contribution of vascular insulin resistance to the early metabolic changes remain unclear. Here, we demonstrate that 3 days of HFD feeding causes endothelial insulin resistance indicated by impaired insulin-induced endothelial NO-synthase (eNOS) activation in adipose tissues and reduced insulin-induced skeletal muscle perfusion. A plasma lipidomic analysis revealed that the levels of C16 ceramide were elevated within 3 days of HFD, and a phosphoproteomic analysis of ceramide-induced phosphorylation in endothelial cells indicated that C16 ceramide induced the phosphorylation of SPARTIN and GABARAPL2, which are involved in lipophagy, a form of autophagy which degrades lipid droplets (LD). Both, exogenous C16 treatment and knockdown of GABARAPL2 in vitro, increased endothelial lipid droplets and inhibited endothelial insulin signaling. In contrast, inhibition of lipid droplet formation blocked ceramide-induced inhibition of endothelial insulin signaling. In vivo, HFD feeding as well as loss of endothelial ATGL, an enzyme for the hydrolysis of triglyceride, increased LD formation and reduced glucose tolerance and insulin sensitivity. Interestingly, C16 ceramide also induced the phosphorylation of TBK1, which is known to regulate the phosphorylation of GABARAPL2 and to thereby disrupt GABARAPL2-mediated lipophagy. Notably, endothelium-specific TBK1 knockout mice show improved glucose tolerance during short-term HFD feeding. Collectively, our findings uncover an endothelial ceramide-TBK1-GABARAPL2 pathway, which regulates endothelial lipid metabolism and affects endothelial and systemic insulin sensitivity during short-term HFD exposure.

A5-3: FAT1 coordinates cardiomyocyte renewal by restraining YAP/TAZ and Notch1 signalling

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The atypical cadherin FAT1 (FAT1) encodes a protocadherin that modulates a variety of downstream pathways, including Wnt/β-catenin and Hippo pathway, which regulate cell proliferation, migration and invasion. Our previous work demonstrated that FAT1 controls degradation of the transcriptional cofactors YAP and TAZ in endothelial cells by interacting with the E3 ubiquitin ligase MIB2, resulting in increased YAP/TAZ protein turnover and inhibitory regulation of endothelial proliferation. YAP/TAZ have been shown to be essential for basal heart homeostasis, and their deficiency exacerbates cardiac injury after myocardial infarction (MI) by reducing cardiomyocyte survival and proliferation. Conversely, activation of YAP/TAZ in cardiomyocytes after MI mitigates myocardial injury, reduces infarct size, improves cardiac function and enhances survival in various animal MI models. Although FAT1 is highly expressed in cardiomyocytes, its role in cardiomyocyte, as well as in cardiac physiology and post-MI recovery, remains unclear. To investigate this, we generated inducible cardiomyocyte-specific FAT1 deficient mice. Following MI, these mice exhibited significantly improved cardiac function, as evidenced by increased ejection fraction and fractional area change compared to the control mice. In vitro, FAT1 knockdown enhanced cardiomyocyte proliferation and reduced apoptosis. Notably, FAT1 loss promoted YAP nuclear translocation in both adult cardiomyocytes and neonatal ventricular myocytes. These findings suggest that cardiomyocyte-specific FAT1 loss exerts a protective effect after MI, via YAP/TAZ activation. However, the precise mechanisms remain to be elucidated. Given the beneficial effects of FAT1 deficiency post-MI, targeting FAT1 or its downstream regulators may represent a novel therapeutic strategy.

A5-4: Clonal hematopoiesis-associated mutations drive pathological intercellular crosstalk in cardiovascular disease

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Introduction: Ageing is a major risk factor for cardiovascular diseases (CVD). Somatic mutations in hematopoietic progenitor cells can confer a proliferative advantage, producing clones of mutant circulating blood cells - a condition known as clonal hematopoiesis (CH), a hallmark of cellular aging. Loss-of-function mutations in DNMT3A are associated with increased inflammation, while splicing factor mutations correlate with myeloid malignancy risk. The molecular impact of CH-mutated immune cells on CVD pathophysiology remains unclear.

Methods and Results: To study DNMT3A CH-driven crosstalk, we integrated scRNA/snRNA-seq data to profile interactions between CH immune cells and resident cardiac cell types using CellChat. DNMT3A-silenced macrophages promoted fibroblast activation via the HB-EGF–EGFR pathway, and their paracrine factors induced cardiac hypertrophy and impaired cardiomyocyte contractility. CH monocytes also interacted with endothelial cells via semaphorin-4A (SEMA4A), upregulated in DNMT3A-silenced macrophages and elevated in patient plasma. Recombinant SEMA4A increased SELE and reduced CDH5 expression in endothelial cells; silencing SEMA4A in DNMT3A-deficient macrophages abolished this effect.

In a second study, mutations in SF3B1, SRSF2, and ZRSR2 in heart failure and aortic stenosis or CAD patients were associated with higher 3.5- or 5-year mortality versus matched controls; SF3B1/SRSF2 CH was further linked to heart failure incidence in the UK Biobank. Paracrine signals from ZRSR2-silenced macrophages induced

cardiomyocyte stress and endothelial dysfunction in vitro and fibrosis in cardiac tissue mimetics; SRSF2 depletion additionally reduced endothelial attachment.

Conclusions: CH-associated mutations in circulating immune cells augment macrophage-to-cardiac cell interactions in CVD.

A5-5: NoxO1 and Nox4 regulate Extracellular Vesicles formation

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Extracellular vesicles (EVs) are increasingly recognized as mediators of inter-organ communication, a fundamental process maintaining physiological homeostasis. The formation and uptake of EVs are two major steps of inter-organ communication. We concentrate on the formation of EVs here. The mechanisms by which reactive oxygen species (ROS)-related proteins influence EVs formation remain incompletely understood.

This study aims to investigate the roles of NOX4 and NoxO1, a subunit of NOX1, in EV formation. HEK293 cells served as a model for donor cells.

HEK293 cells were acutely transfected to overexpress eGFP together with NoxO1, with NoxO1 predominantly localized to the plasma membrane and cytosol, or LacZ together with NOX4, with NOX4 localized to subcellular organelles including the endoplasmic reticulum, Golgi apparatus, and nuclear membrane. Extracellular vesicles (EVs) were isolated using ultracentrifugation and characterized by western blotting(WB), transmission electron microscopy (TEM), and nanoparticle tracking analysis (NTA). WB showed that EVs were detected by their marker protein TSG101, Alix, and CD9, with no significant differences in expression levels among Hek-eGFP and Hek-NoxO1, Hek-LacZ and Hek-NOX4, or between Hek-NoxO1 and Hek-NOX4 groups. NTA revealed that EVs derived from HEK293 cells were predominantly distributed below 200 nm in diameter. Compared with negative control groups, NoxO1 overexpression increased the number of EVs in the 101–200nm size range, whereas NOX4 overexpression led to an increase in EVs within the 1–100 nm size range. The same three characterization methods were also applied to EVs isolated from human plasma under physiological conditions, besides, TEM and WB were applied to characterize plasma-derived EVs from mice under physiological conditions. WB indicated that EVs were detected by Alix and TSG101, with TSG101 exhibiting markedly higher expression than Alix.

A5-6: Human Endogenous Retroviral Resurrection as a Driver of Cardiac Aging and Disease

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Transposable elements (TEs), including human endogenous retroviruses (HERVs), constitute nearly half of the human genome. Far from inert genomic remnants, these loci function as a sophisticated regulatory layer with potent promoter and enhancer capabilities. In healthy tissues, TEs are silenced by KRAB zinc finger proteins (ZNFs), which recruit the KAP1 (TRIM28) machinery to enforce heterochromatin repression. However, the specific regulatory grammar, governing which ZNFs control which TEs in distinct cell states remains a fundamental blind spot in our understanding of cardiovascular health. We propose that the breakdown of this ZNF shield is a primary driver of cardiovascular aging. During senescence, the deterioration of heterochromatin unlocks specific TE and HERV subsets, triggering viral mimicry and innate immune activation. This is not a passive byproduct of age. It is a structured, causal amplifier of pathological remodeling that erodes the identity of endothelial cells and cardiomyocytes. Our preliminary data supports this model. Repeat-aware analysis of aged cardiovascular cells reveals: selective activation of evolutionarily recent HERVs, chromatin accessibility gains at specific repeat loci, shifts in ZNF motif activity and heterochromatin reorganization, induction of cytosolic nucleic acid sensing and inflammatory signaling. This project will establish the first mechanistic framework linking reactivated repeats, their ZNF controllers and the downstream pathways destabilizing cardiac homeostasis. By creating a cardiopulmonary atlas of TE resurrection and decoding the cell-state-specific ZNF grammar, we will transform the repeat-encoded genome from an annotated backdrop into a testable driver of disease. This work will uncover foundational principles of cardiac aging and identify new therapeutic intervention points to restore repeat control and rewire pathological inflammatory states.

A5-7: Endothelial cell-derived SMOC1 deficiency, limits cardiac remodelling after myocardial infarction

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Endothelial-to-mesenchymal transition (EndMT) describes a transforming growth factor (TGF) -β) process in which endothelial cells acquire mesenchymal features. The switch is activated after myocardial infarction (MI) and EndMT cells participate in cardiac tissue repair, impact on infarct size and contribute to fibrosis and adverse remodelling. We screened available single cell sequencing datasets for TGF-□ regulated genes activated after acute MI in mice. A cluster of endothelial cells expressing mesenchymal markers expressed secreted modular calcium binding protein 1 (SMOC1), which was previously reported to bind to the TGF-□ accessory receptor; endoglin. In cultured human endothelial cells, EndMT induction (IL-1β, TGF-β2) upregulated SMOC1. SMOC1 silencing via siRNA enhanced EndMT markers (smooth muscle actin, endoglin, S100A4) and reduced endothelial cell phenotypic markers such as VE-cadherin. Also, the TGF-□2-induced phosphorylation of SMAD2, was attenuated in SMOC1-deficient cells indicating that TGF-□2 signalling was regulated. The addition of recombinant SMOC1 largely prevented all of these changes and had a marked impact on endothelial cell gene expression such as an increase in the expression of endothelial phenotypic markers like VE-Cadherin. To assess the impact of endothelial cell-derived SMOC1 in vivo VE-cadherin-cre and SMOC1fl/fl mice were crossed to generate animals lacking SMOC1 in endothelial cells. These SMOC1□EC mice were then subjected to MI using a minimally invasive surgical approach. The lack of endothelial cell SMOC1 amplified the inflammatory response 1 and 3 days after MI, as evidenced by a marked increase in the cardiac recruitment of macrophages, monocytes and T cells, in particular CD4+ T cells. Functional analyses after MI revealed a continual worsening of cardiac function in the SMOC1□EC mice compared to their wild-type littermates with markedly increased end diastolic volume (WT: 72.6 ± 7.5 µL vs. SMOC1: 134.8 ± 14.4 µL) and substan

A5-8: SARS-CoV-2 promotes an unstable proinflammatory atherosclerotic plaque phenotype

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Viral respiratory infections, like SARS-CoV-2, are associated with an elevated long-term risk for stroke and myocardial infarction. Although effects of SARS-CoV-2 on atherosclerotic plaque development have been proposed, mechanistic insights and evidence remain limited.

In atherosclerotic mice (LDLR-/- on a high-fat diet), we observed features of plaque instability (fibrous cap/necrotic core ratio) 28 days after SARS-CoV-2 infection (1000 PFU of murine-adapted SARS-CoV-2), while plaque size remained unchanged. To gain molecular insights into how SARS-CoV-2 affects the composition and phenotype of atherosclerotic plaques, we performed scRNA-seq of plaques 28 days after SARS-CoV-2 infection. Here, we found a proinflammatory plaque phenotype characterised by enrichment of immune cells. Specifically, numbers of recruited Mhc-II+/Ccr2int macrophages and dendritic cell (DC) subtypes (cDC1, CCR9+ DCs and mature) were increased, while resident macrophage numbers were unchanged. In addition, we found increased numbers of T-cell subsets (Naive Cd4+, T-regs, Naive Cd8+, and Il17+Cxcr6+). Investigating the transcriptomic profile of plaque immune cells revealed that proinflammatory cytokines were significantly upregulated in macrophages (Ccl2, Cxcl10, IL-1α), dendritic cells (Ccl5, Cxcl9), and T cells (TNF and TNF superfamily members). In addition, recently described immunoregulatory endothelial cells were induced, which express major histocompatibility complex class II genes and may present antigens to T cells, thereby facilitating their recruitment to the vessel wall. Ligand-receptor analysis revealed increased recruitment signalling to macrophages, monocytes, dendritic cells and T-cells via the CXCL12-CXCR4 axis, which might be a potential therapeutic target. Our data dissect the in vivo effects of SARS-CoV-2 on the atherosclerotic vasculature, providing mechanistic insights into how SARS-CoV-2 infection affects the instability of atherosclerotic plaques.

A5-9: SARS-CoV-2 infection promotes cardiac remodelling and dysregulation of homeostatic cardiac macrophages

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After viral respiratory infections, like SARS-CoV-2, the risk for long-term cardiovascular complications, including arrhythmias, autonomic dysfunction and heart failure, is increased. Patients with cardiometabolic disease are particularly vulnerable. Mechanistic insights into how viral infections contribute to the development of cardiovascular pathology and post-acute sequelae remain limited.

We found that 28 days after SARS-CoV-2 infection (1000 PFU murine-adapted SARS-CoV-2), mice with concomitant cardiometabolic disease (LDLR-/- + high-fat diet) exhibit amplified cardiac remodelling, including cardiac fibrosis and reduced cardiac innervation, compared to C57BL/6 mice. When examining the immune cell repertoire of the heart after viral infection, we found no increase in proinflammatory recruited myeloid immune cells at either the acute stage or after convalescence. Instead, the number of cardiac tissue-resident macrophages in cardiometabolically preconditioned animals dropped significantly during acute infection. Despite partial recovery of this protective macrophage subset, its levels remained markedly reduced at 28 days post-infection. To gain molecular insights into the cellular landscape of the heart following viral injury, we performed single-nuclei RNA sequencing of cardiac tissue 28 days after SARS-CoV-2 infection. In cardiac resident macrophages, we observed that proinflammatory gene ontology (GO) signatures were upregulated (GO-terms: “antigen processing and presentation of exogenous peptide”, “positive regulation of immune response”). Notably, not only were proinflammatory pathways enriched, but genes associated with homeostatic function were downregulated, as reflected by the downregulated GO-terms “blood vessel development”, “heart development” and “axon guidance”. No replicable virus or viral RNA could be detected in the heart. Our data suggest that dysregulation of resident macrophages could mediate cardiac complications following viral infection.

A5-10: Effect of microplastic tire abrasion of endothelial angiogenic capacity

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Urbanization and increasing traffic have led to continuous emissions of traffic-related contaminants. Tire and road wear particles in road runoff are of particular concern due to their potential contribution to long-term human exposure. Cardiovascular disease is the leading cause of death world-wide and atherosclerosis, inflammatory cell activation as well as failure of angiogenesis contribute to this. We hypothesize that road runoff impacts the function of endothelial cells, which are critical to maintain vascular health.

Road runoff samples were collected over two years from the A4 highway in Aachen (Germany). Samples were filtered and organic extracts were produced from the filter residues. Freeze-dried samples were sterilized by gamma irradiation and extracted using ultrasound-assisted extraction. In vitro, particulate filter residues, organic extracts, and the particles washed after extraction were examined on their effect on human umbilical vein endothelial cells (HUVEC). The following aspects were studied: proliferation, apoptosis assays and gene expression as determined by quantitative PCR, focusing on markers for oxidative stress, immune responses, and endothelial-associated genes.

Road runoff induced concentration-dependent effects on apoptosis and proliferation of endothelial cells. In addition, proinflammatory transcriptional responses were detected (CD31, IL-6, ANGPTN2), indicating activation of stress- and immune-associated signaling pathways. The strongest effects occurred in particle-containing fractions in comparison to the particle free organic extracts (IC50 = 2.1 mg/ml versus IC50 = 11.2 mg/ml), suggesting that particle-mediated processes contribute significantly to the induction of endothelial dysregulation. The results will contribute to a better understanding of the human toxicological relevance of traffic-related runoff and provide insights into the processes through which complex environmental mixtures can influence endothelial functions.

A5-11: Functional Characterization of Mitochondrial Protein NDUFA4L2 in Pulmonary Arterial Smooth Muscle Cells Exposed to Chronic Hypoxia and Pulmonary Hypertension

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Introduction: Pulmonary hypertension (PH) is caused by vascular remodelling of the pulmonary arteries (PA) induced by resistance to apoptosis and hyperproliferation of pulmonary arterial smooth muscle cells (PASMC). This phenotype is sustained by decreased mitochondrial respiration (MR) and increased anaerobic glycolysis, leading to a metabolic adaptation (MA). The mitochondrial NADH dehydrogenase 1a subcomplex subunit 4-like 2 (NDUFA4L2) is upregulated in several cell types exposed to chronic hypoxia (CH); however, its role in the MA of the PASMC exposed to CH and PH remains unclear. Materials and methods: RNAseq, western blot (WB) and immunostaining of the pulmonary arteries (PA) were used to quantify NDUFA4L2 in mice exposed to CH (10% O_2) and in lung biopsies of patients with idiopathic PH (IPAH). qPCR and WB were used to detect NDUFA4L2 expression in isolated mouse and human PASMC exposed to CH (1% O_2), and high-resolution respirometry to examine MR. The role of NDUFA4L2 in PASMC during CH was tested by siRNA-mediated downregulation, followed by cell proliferation and scratch assays, and WB to explore proliferative (pAKT, pERK), apoptotic (BAX, cleaved PARP, cleaved caspase-3) and metabolic (PDHK1, LDHA, PC) and OXPHOS markers. Results: NDUFA4L2 protein was upregulated in the lung homogenate and PA of mice exposed to CH, and in the lung biopsies from IPAH patients. In vitro, NDUFA4L2 mRNA and protein were upregulated in mouse and human PASMC, along with decreased MR. Silencing NDUFA4L2 in PASMC exposed to CH decreased cell survival and wound healing, increased protein levels of apoptotic markers and restored mitochondrial complex I and III subunits, and pyruvate carboxylase. Conclusion: Our results show a direct role of NDUFA4L2 in the pathogenesis of CH-induced PH. It controls mitochondrial function, cellular survival by resisting apoptosis of the PASMC during CH. Future investigations using NDUFA4L2 KO mice will clarify its role in the pathophysiology of PH.

A5-12: Dermcidin: A Novel Antimicrobial Peptide Driving Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a progressive disorder characterized by elevated pulmonary vascular resistance due to pathological remodeling of the pulmonary vasculature, leading to right heart failure. Current therapies provide symptomatic relief without targeting disease mechanisms, underscoring the need to identify novel molecular drivers. Using an unbiased proteomic screening from membrane proteins, we identified dermcidin (DCD), an antimicrobial and stress-response protein, as significantly upregulated in IPAH pulmonary artery smooth muscle cells (PASMCs). This was validated at mRNA and protein levels in PASMCs and lung homogenates from PAH patients and in a monocrotaline rat model. DCD was further increased in IPAH PASMCs after stimulation with PDGF-BB, TNF-α, IL-6, and H_2O_2, indicating responsiveness to proliferative, inflammatory, and oxidative stress. DCD acts as a pro-inflammatory mediator regulating oxidative stress, cytokine signaling, and endothelial dysfunction and has been implicated in cancer and ischemia. In vivo, recombinant DCD induced cardiomyocyte injury and oxidative stress in mice, resembling myocardial injury under metabolic stress. Clinically, circulating DCD was elevated in male PAH patients and negatively correlated with cardiac index, while no change was seen in females, suggesting sex-specific involvement. DCD expression was also increased in right ventricles of male patients with decompensated RV hypertrophy and negatively correlated with cardiac index. Pharmacological inhibition of DCD using seriniquinone reduced PASMC proliferation and induced apoptosis. Mechanistically, DCD inhibition decreased ERK and STAT3 phosphorylation and downregulated Cyclin D1, indicating MAPK/STAT pathway involvement. Ongoing studies assess DCD in vascular remodeling, RV failure, and systemic inflammation. Collectively, these findings identify DCD as a novel contributor to PAH pathogenesis and a potential therapeutic target.

A5-13: Molecular Mechanisms of Defective Viral Genome Formation and Immune System Interaction in Influenza Viruses

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Highly pathogenic avian influenza viruses (HPAIVs) occasionally cross the species barrier, followed by excessive host inflammation. Their high genetic variability complicates pandemic risk assessment.

Deletion-containing viral genomes (DVGs) arise through internal deletions during error-prone Influenza A virus (IAV) replication and compete with full-length viral RNAs for polymerase complexes to suppress viral replication. DVGs are packaged into viral particles and provide immune ligands for pattern recognition receptors (PRRs), which may promote inflammation in HPAIV infections, contributing to the severe immunopathology in humans.

We hypothesize that virus-specific DVG landscapes determine IAV pathogenicity and could serve as diagnostic markers for rapid pandemic risk assessment. By characterizing these signatures across seasonal and highly pathogenic IAV strains, this study aims to establish a framework for the DVG-based antiviral, immunomodulatory, and diagnostic strategies.

Long-read sequencing showed that DVG-landscapes are strain-specific, with mvRNAs detected only in HPAIV infections. Results from PRR-immunoprecipitations revealed that selected HPAIV-derived mvRNAs act as potent PKR-specific immune activators, potentially driving host inflammation. Furthermore, distinct RNA secondary structures and selective DVG packaging suggest that specific structural motifs govern both their encapsidation and immune recognition.

In this study, we envision unraveling the molecular determinants of DVG formation, characterize DVG-specific RNA structural elements, and dissecting the role of the viral polymerase complex in DVG biogenesis. It further investigates DVG-driven immune activation, and finally, the therapeutic potential of engineered DVGs and mvRNAs as antiviral and immunomodulatory tools. We will translate our approach into more complex and native human lung models to assess their capacity to suppress IAV replication or selectively stimulate protective immunity.

A5-14: PERK inhibition rewires translational and CMGC protein kinase networks into an antiviral state

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Despite the success of protein kinase inhibitors in oncology, fewer than 5% of human kinases are therapeutically targeted, and none have been approved for infectious diseases, including RNA virus infections. A major challenge in developing kinase-based antivirals is understanding the complex and dynamic interactions between kinases and their substrates during cellular perturbation. In this study, we investigated how inhibition of a single kinase globally alters the state of virus-infected cells. By targeting PERK, a central sensor of coronavirus-induced ER stress, we show that pharmacological inhibition reshapes host translational and kinase signaling networks toward an antiviral phenotype. We integrated transcriptomic, translatomic, proteomic, and phospho-proteomic datasets from Huh7 cells infected with human coronavirus 229E under PERK inhibition. Bioinformatic analysis revealed a previously unrecognized connection between PERK and the Rho GTPase–PAK signaling axis. Inhibition of PAK kinases resulted in potent antiviral effects, highlighting the therapeutic potential of targeting kinase network rewiring. A key aspect of this work is the application of the human kinome motif atlas, comprising 89,000 phosphorylation sites and 303 serine/threonine kinase motifs, to evaluate PERK inhibition effects. This analysis showed that coronavirus infection co-regulates entire kinase families rather than individual enzymes, providing a systems-level view of kinome reorganization. Further analysis of infected cells, supported by re-analysis of SARS-CoV and SARS-CoV-2 datasets, identified a conserved CMGC kinase module and more than 140 substrates activated by coronaviruses and suppressed by PERK inhibition. Overall, these findings establish a framework for exploiting kinase network rewiring to induce antiviral states and support kinase inhibitors as anti-infectives.

A5-15: The role of iNOS in the hyperoxia-induced mouse model of bronchopulmonary dysplasia

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RATIONALE: Bronchopulmonary dysplasia (BPD) is a chronic lung disease of preterm infants, driven by oxygen toxicity and characterized by alveolar simplification and abnormal pulmonary vasculature. Given the role of inducible nitric oxide synthase (iNOS/Nos2) in smoke-induced emphysema models, we hypothesized that iNOS contributes to hyperoxia-induced alveolar injury in a murine BPD model.

METHODS: Newborn C57BL/6J WT and Nos2-/- mice were exposed to room air (21% O_2) or hyperoxia (85% O_2) from postnatal day (P)1 to P14. At P14, echocardiography, forced oscillation technique, micro-computed tomography, design-based stereology, and assessment of elastin (Eln) and iNOS gene and protein expression in lung tissue were performed. Nos2 expression was assessed in M1/M2 bone marrow-derived macrophages exposed to 85% O_2 for 24 h. iNOS protein and 3-nitrotyrosine were measured in M2 macrophages after hyperoxia.

RESULTS: Hyperoxia-exposed Nos2-/- mice showed improved cardiac function and lung structure, with increased tissue density, decreased air volume, thinner septa and higher alveolar density, along with partially restoration of lung compliance. Nos2 mRNA was increased in hyperoxia-exposed WT lungs. In M2 macrophages, hyperoxia similarly induced Nos2 expression and 3-nitrotyrosine formation, indicating increased nitrosative stress, which was attenuated in Nos2-/- cells. No changes were observed in M1 macrophages. Eln expression and protein levels, reduced in hyperoxic WT mice, were restored in Nos2-/- mice to levels comparable to normoxic WT controls.

CONCLUSIONS: Nos2 deletion in hyperoxia-exposed mice improved cardiac and pulmonary function and structure, suggesting increased iNOS contributes to impaired lung development. Reduced 3-nitrotyrosine in Nos2-/- M2 macrophages supports their causal role in BPD. Targeting iNOS may represent a therapeutic strategy.

A5-16: Cyclophilin D Deficiency Triggers Spontaneous Development of Pulmonary Hypertension and Emphysema during Aging

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Background: Pulmonary hypertension (PH) associated with chronic obstructive pulmonary disease (COPD) leads to increased morbidity and mortality. Mitochondrial cyclophilin D (CypD), encoded by the peptidylprolyl isomerase F (Ppif) gene, regulates the mitochondrial permeability transition pore and may contribute to PH-COPD by modulating apoptosis. Previously, we found that CypD modulates pulmonary vasoconstriction. Therefore, in this study, we investigated the role of CypD in PH-COPD development.

Methods and Results: Wild-type (WT) and CypD knockout (Ppif-/-) mice (aged 3 months) were exposed to cigarette smoke (CS) or room air (RA) for 8 months (6 hours/day, 5 days/week). Interestingly, RA-exposed Ppif-/- mice (now aged 11 months) exhibited signs of PH compared to RA-exposed WT mice (also aged 10-11 months), characterized by increased right ventricular systolic pressure (RVSP) and right ventricular hypertrophy. At 3 months, RVSP was similar between WT and Ppif-/- groups. Additionally, 8-month RA-exposed Ppif-/-mice showed signs of emphysema compared to RA-exposed WT mice, as indicated by in vivo lung function tests, and post-mortem stereological analysis. However, after 8 months of CS exposure, Ppif-/- mice developed PH and emphysema to a similar extent as WT mice. Single cell sequencing analysis of mice and human lungs shows that Ppif it is highly express in AEC2 cells and in monocytes. Moreover, CypD deficiency increased the expression of the endoplasmic reticulum (ER) stress marker X-box binding protein 1 (XBP1) and the cell cycle arrest marker p21 in the lung homogenates of 3-month-old mice, while it reduced cyclin D1 expression in 12-month-old mice compared to WT mice of the same age.

Conclusion: CypD deficiency promotes pulmonary alterations resembling PH and emphysema during aging but does not exacerbate these conditions following CS exposure. Inhibition of cellular proliferation leading to parenchymal changes may underlie these alterations.

A5-17: Staphylococcus aureus exploits the lung environment to cause persistent infections

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Staphylococcus aureus is a dangerous human pathogen associated with one million annual deaths. The bacterium utilizes its arsenal of virulence factors and strategies to cause versatile infections including endocarditis, septicemia and pneumonia. Pulmonary staphylococcal infections are especially problematic in people suffering from the hereditary disease cystic fibrosis (CF). This recessive genetic disorder is characterized by thick airway mucus and a decreased mucociliary clearance, which predisposes people with CF to chronic lung inflammation and infection. In the CF lung, S. aureus frequently develops small colony variants (SCVs) that have a reduced growth rate and an increased resistance phenotype. Despite their dependency on nutrients of low pulmonary abundance, these bacterial variants persist and cause recurring infections. Here, we investigated a mechanism by which S. aureus can independently survive in the lung microenvironment. Biochemical studies and growth models demonstrated the ability and relevance of specific microbial determinants to support bacterial viability. Confirmed by tissue culture experiments and a model of murine pneumonia, we identified this mechanism as a crucial survival strategy for S. aureus SCVs. Overall, our findings may aid in the discovery of alternative treatment options to eradicate staphylococcal SCVs during pulmonary infections.

A5-18: Role of distinct nucleotide-modulating enzyme in S. aureus

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S. aureus is Gram-positive bacterium colonizing over 30% of the human population. Due to the large-scale use of antibiotics, an increasing amount of methicillin-resistant S. aureus (MRSA) emerges and directly imposes heavier clinical burden. S. aureus can secrete a broad spectrum of virulence factors and structural components to module multiple mechanisms, facilitating its survival and infections at the host-pathogen interaction surface. Within the large staphylococcal secretome, some enzymes are directly associated with signaling nucleotides and therefore potentially relevant to crucial virulence mechanisms. Intensive studies have identified several signaling molecules in bacteria, including S. aureus. Although some S. aureus clones harbor the relevant enzymes that generate specific nucleotides, their actual functions remain unknown. Therefore, this project aims to study whether the nucleotide-synthesizing enzymes in S. aureus contribute to bacterial survival or infections, and whether the functions can directly interfere with clinical treatments. Our data indicate that the candidate enzyme in S. aureus play a crucial role in the adaptation of this microbe and further regulate the interaction with non-staphylococcal organisms.

A5-19: Precision Repair: Ligand-Specific Lymphotoxin β Receptor Signaling in Influenza Pneumonia

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Severe viral infections caused by pathogens such as influenza A virus (IAV) or SARS-CoV-2 can cause lasting structural changes in the lung through remodeling processes governed by incompletely understood signaling pathways. Lymphotoxin beta (LTα1β2) and tumor necrosis factor superfamily member 14 (TNFSF14) are known mediators of tissue remodeling in chronic lung diseases such as asthma, pulmonary fibrosis, and COPD via their shared receptor, lymphotoxin beta receptor (LTβR), yet any involvement in post-viral lung remodeling currently remains unclear. To address this, we established an IAV-induced injury microenvironment using a murine bronchoalveolar lung organoid (BALO) model. Treatment with bronchoalveolar fluid collected from wild-type (wt) mice at day 8 post- infection (pi) significantly upregulated LTβR expression in both epithelial and mesenchymal compartments. BALO stimulation with recombinant TNFSF14 markedly increased alpha-smooth muscle cell actin (aSMA) expression and pro-inflammatory cytokine release, while LTα1β2 elicited only a mild response, indicating ligand-specific mesenchymal activation. Single-cell RNA sequencing of lungs from IAV-infected wt mice revealed a cellular interaction network characterized by neutrophil-derived TNFSF14 and increased LTβR expression across multiple structural cell types during the repair phase after infection, including venous endothelial cells, transitional alveolar epithelial cells, and peribronchial fibroblasts. LTβR-deficient mice exhibited reduced weight loss and increased numbers of epithelial cells at day 14 pi compared to wt controls, despite similar levels of epithelial cell loss at day 7 pi. These findings suggest an enhanced epithelial regenerative capacity upon disruption of LTβR signaling. Together, our data identify a myeloid–structural cell signaling axis as a key regulator of post-IAV lung repair and highlight LTβR as a potential therapeutic target to promote recovery following viral lung injury.

A5-20: Lung tumor–infection niche drives macrophage plasticity and tumor suppression

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Macrophage plasticity critically shapes tumor progression, yet the signals that reprogram pro-tumor macrophages within the lung microenvironment remain incompletely defined. Here, we show that infection-derived cues drive a phenotypic switch toward tumor suppression. Exposure of pro-tumor macrophages to bronchoalveolar lavage fluid (BALF) from Influenza A virus (IAV)-infected lungs reprogrammed these cells into a tumor-inhibiting state, with maximal activity observed at day 7 post-infection. Proteomic profiling of day 7 IAV BALF identified Serum Amyloid A4 (SAA4) as a key secreted factor mediating this effect. Recombinant SAA4 was sufficient to induce anti-tumor macrophage polarization in both human and mouse systems. Transcriptomic analysis further revealed induction of the necroptosis effector Mixed Lineage Kinase Domain-Like protein (MLKL) in reprogrammed macrophages. Functional perturbation demonstrated that MLKL is required for acquisition of the pro-inflammatory, tumor-suppressive phenotype and acts in concert with SAA4. SAA4-reprogrammed macrophages suppressed tumor cell proliferation and induced apoptosis in human and murine tumor-derived precision lung slices (TD-PCLS) / tumor-cell-seeded PCLS (TCS-PCLS), and significantly inhibited tumor growth in orthotopic lung cancer models. In contrast, genetic or functional ablation of SAA4 or MLKL enhanced tumor progression and promoted accumulation of anti-inflammatory macrophages within the tumor microenvironment, as confirmed by multiplex imaging and single-cell transcriptomics (scRNAseq). These findings define an infection-induced SAA4–MLKL axis that reprograms macrophage fate and restrains lung tumor growth, highlighting a therapeutic strategy to harness innate immune plasticity for cancer control.

A5-21: Cell-Type-Specific Mechanisms of Pulmonary Vascular Alterations in Cigarette Smoke-Induced COPD

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Cigarette smoke (CS)-induced nitrosative and oxidative stress causes pulmonary vascular alterations and leads to pulmonary hypertension (PH) in chronic obstructive pulmonary disease (COPD). Although increased levels of key stress-related enzymes, such as inducible nitric oxide synthase (iNOS) and NADPH oxidase organiser 1 (NOXO1), have been reported in remodeled pulmonary vessels, the underlying cell-specific mechanisms remain poorly understood. We used in vivo models of chronic CS exposure in mice with conditional deletion of stress sources (iNos or Noxo1) in endothelial cells ($Tie2^+$) or α-smooth muscle actin-expressing cells ($Acta2^+$) along with transcriptomic profiling of human COPD lungs and mouse pulmonary vessels. Endothelial cell-specific deletion of iNos/Noxo1 ameliorated CS-induced proliferation of pulmonary artery smooth muscle cells (PASMCs) and pulmonary vascular alterations, and was associated with decreased levels of inflammatory cytokines, including tumor necrosis factor α (TNFα). In vitro validations in human confirmed that these cytokines promote PASMC proliferation. Conversely, deleting iNos/Noxo1 in $Acta2^+$ cells did not prevent vascular remodeling. Instead, lowering nitrosative/oxidative stress in these cells increased PASMC proliferation, accompanied by increased E2F1 activity and reduced expression of contractile markers. Consistent with the mouse models, this E2F1-related signature was also observed in patients with COPD, which showed significant vascular remodeling and low levels of local stress markers. These results support a multi-hit model for COPD-related vascular changes, comprising nitrosative/oxidative stress, an inflammatory phase driven by endothelial cell-derived cytokines, possibly followed by and exacerbated by an E2F1-driven hyperproliferative PASMC state. Consequently, therapeutic interventions targeting nitrosative/oxidative stress in COPD require cell-type-specific precision to avoid unintended worsening of vascular pathology.

A5-22: Towards Safer Lung-Targeted Antiviral Prophylaxis: Exploring Functional Heterogeneity of Human IFN-α Subtypes in Pulmonary Endothelial Cells through Single Cell Transcriptomics

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Type I Interferons (IFNs), particularly IFN-α subtypes, are promising candidates for lung-localized antiviral prophylaxis, although their cell type-specific effects remain insufficiently characterized, limiting safe clinical translation. Systemic IFN-α2/-β administration has been associated with Pulmonary Hypertension (PH), underscoring the need to identify subtypes that preserve vascular homeostasis during localized delivery. We evaluated the functional heterogeneity of selected IFN-α subtypes by single cell sequencing in human lung explants. Across 21 cell-types, including 7 Pulmonary Endothelial Cell-types (PECs), we mapped Interferon-Stimulated Gene (ISG) signatures within the lung microenvironment. Pulmonary Arterial Endothelial Cells (PAECs) and Pulmonary Vascular Smooth Muscle Cells (PVSMCs) were prioritized for deeper analysis due to their key roles in vascular remodeling and PH pathogenesis. Exposure to IFN-α subtypes induced markedly divergent transcriptional programs across these PECs: PAECs exhibited strong induction of antiviral ISGs like MX1, IFITs, etc, alongside proinflammatory cytokines like IL6, IL10, etc, while PVSMCs showed more attenuated and subtype-specific ISG responses with limited engagement of inflammatory cytokines. Notably, IFN exposure also activated vasoconstrictive programs in a subtype-specific manner, where the strongly antiviral subtypes suppressed factors like VEGF1, proving to be promising candidates for further clinical investigation. These findings demonstrate that IFN-α subtypes are functionally non-redundant, and elicit cell type-specific antiviral, inflammatory, and vascular responses, enabling rational selection of candidates that maximize antiviral efficacy, with minimal endothelial dysfunction, vascular toxicity and PH risk.

A5-23: Ubiquinol–cytochrome c reductase hinge protein deletion selectively impairs acute hypoxic pulmonary vascular responses

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Mitochondria are key regulators of pulmonary arterial responses to hypoxia. Complex III–derived mitochondrial reactive oxygen species (mtROS) drive acute hypoxic pulmonary vasoconstriction (HPV) but are reduced in chronic hypoxia–induced pulmonary hypertension (PH). Ubiquinol–cytochrome c reductase hinge protein (Uqcrh) is functionally essential for complex III. However, the role of Uqcrh in HPV and PH remains unclear.

Uqcrh$^{-/-}$ and wild-type mice, together with their pulmonary arterial smooth muscle cells (PASMCs), were studied. Pulmonary arterial pressure, membrane depolarisation, intracellular calcium dynamics, mtROS, respiration and coenzyme Q levels were assessed under acute hypoxia. Uqcrh expression, cell proliferation and HIF-1α levels were evaluated during chronic hypoxia.

Uqcrh$^{-/-}$ impaired acute hypoxia–induced HPV, membrane depolarisation, calcium signalling, mtROS, and coenzyme Q oxidation, but did not alter cell proliferation or HIF-1α elevation during chronic hypoxia. Uqcrh expression was not regulated by chronic hypoxia. Thus, Uqcrh is involved in acute but not chronic oxygen sensing.

A5-24: Extracellular vesicles from Klebsiella pneumoniae facilitate bacterial pneumonia in intubated patients by impairing the bactericidal properties of alveolar macrophages

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RATIONALE

Nosocomial pneumonia frequently affects intubated patients and is strongly associated with airway colonization by Klebsiella pneumoniae. However, the mechanisms linking colonization to infection remain poorly defined. Bacterial extracellular vesicles (bEVs) released by K. pneumoniae contain diverse bioactive cargo capable of modulating host responses. Because alveolar macrophages (AMs) are critical for bacterial clearance, we hypothesized that K. pneumoniae–derived bEVs impair AM bactericidal function and thereby promote pneumonia development.

METHODS

K. pneumoniae was cultured and exposed to subinhibitory antibiotic concentrations before isolation of secreted bEVs. Murine and human AMs were obtained by bronchoalveolar lavage and stimulated with bEVs. Bactericidal activity was assessed after infection with viable K. pneumoniae. Reactive oxygen species (ROS) were quantified by flow cytometry, cytokines by multiplex assay. Mitochondrial dynamics were analyzed using Leica confocal microscopy.

RESULTS

Preexposure to bEVs reduced the bactericidal capacity of AMs and facilitated bacterial outgrowth following intratracheal bEV instillation and subsequent K. pneumoniae infection. bEVs suppressed mitochondrial ROS (mtROS) generation and reduced cellular respiration in both murine and human AMs. Protein inactivation in permeabilized bEVs restored mtROS production and bacterial killing, whereas nucleic acid degradation had no effect. Notably, bEVs isolated from colonized patients similarly suppressed mtROS production and promoted mitochondrial fusion.

CONCLUSIONS

K. pneumoniae–derived bEVs suppress antimicrobial responses in alveolar acrophages, impairing bacterial clearance and potentially driving progression from airway colonization to pneumonia.

A5-25: Mitochondrial Cytochrome c Oxidase Subunit 4 Isoform 2 Regulates Neutrophilic Inflammation during Cigarette Smoke- and Influenza Virus-induced inflammation.

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Chronic obstructive pulmonary disease (COPD), mainly caused by inhalation of noxious gases such as cigarette smoke (CS), is a severe, incurable lung disorder that lacks targeted therapies. CS increases susceptibility to viral infections and exacerbations. Mitochondrial reactive oxygen species (mtROS) regulate immune responses and might serve as a potential therapeutic target. Here, we identify mitochondrial cytochrome c oxidase subunit 4 isoform 2 (COX4I2), a mediator of mtROS release, as crucial for smoke- and virus-induced inflammation. Cox4i2$^{-/-}$ mice exhibited significantly lower levels of inflammatory cytokines, CXCL1 and IFNγ, after CS and infection, along with reduced neutrophils in bronchoalveolar lavage. Single-cell RNA sequencing revealed that Cox4i2-high-expressing pericyte subsets regulate neutrophil migration in the lung. This indicates that Cox4i2-dependent mtROS in pericytes drives neutrophilic inflammation during CS, highlighting it as a promising therapeutic target in COPD

AREA 6

A6-1: Investigating the specification and maturation of the cardiac pacemaker

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Cardiac arrhythmia, caused by abnormal heart pacing, affects up to 5% of the population. The cardiac conduction system plays a crucial role in generating electrical impulses and ensuring rhythmic contraction. Although several key players involved in the development of the cardiac pacemaker, the sinoatrial node (SAN), have been identified, little is known about the proteins and signaling pathways that lead to SAN maturation. This project aims to identify genes and signaling pathways affecting SAN formation and maturation while also modeling arrhythmias and aiding in the development of therapeutic interventions. We have identified using single-cell RNA sequencing data from zebrafish hearts across different time points, several candidate genes that are upregulated in the SAN region during early embryonic stages (after heart looping at 36 hours post-fertilization (hpf)). These SAN genes exhibit a high degree of conservation from zebrafish to humans. We then performed a crispant (i.e., mosaic crispr) screen to identify genes that when mutated would lead to changes in cardiac contraction (heart rate, fractional shortening) for further evaluation using stable mutant lines. Among these candidate genes is sema3aa (semaphoring-3a), an axonal chemorepellent, which is important for cardiac innervation. To study its function, we have generated a mutant model for this gene, and our preliminary data indicates a hyperinnervation phenotype accompanied by a reduction in heart rate. Another candidate, fgf13a (fibroblast growth factor 13a), is highly expressed in the SAN region and is known to regulate Na+ channel activity. Using stable mutant and mosaic overexpression model we show that fgf13a can regulate the number of shox2 +ve cells in the heart, thereby regulating the specification of the cardiac pacemaker. We hypothesize that these genes play a crucial role in driving SAN maturation, establishing a stable heartbeat, and coordinating cardiac–neural regulation.

A6-2: Investigating the Role of Wnt/β-catenin Signaling in Immune Cell Infiltration Across the Blood-Brain Barrier

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Tight control of vascular permeability is essential for the homeostasis and functionality of the central nervous system (CNS), which is maintained by the blood-brain barrier (BBB). The Wnt/β-catenin pathway is known to regulate barrier function of endothelial cells at the BBB. In the adult brain, astrocytes (ACs) are the major source of Wnt growth factors. Moreover, ACs are the cell of origin of aggressive glioma brain tumors, in which the BBB is substantially impaired, and therapeutic activation of endothelial β-catenin signaling rescues BBB function and reduces tumor growth. Given that BBB impairment and glioma growth go along with substantial inflammatory reactions, we hypothesize that the Wnt/β- catenin pathway may also influence the inflammatory response.

To investigate this, we make use of a mouse model with a mild impairment of BBB function, in which we conditionally deleted the Evi gene in ACs, which is essential for Wnt release (GFAP-Cre:Evi lox/lox = AC ΔEvi). We performed staining for inflammatory cells (CD45, CD11b, CD3) and analyzing for extravasation in mouse brain sections of = AC ΔEvi and control mice.

Similarly, we started to analyze GL261 mouse glioma models, in which Wnt signaling was manipulated through Wnt1 overexpression, endothelial-specific activation (AAV-Wnt7- agonist), and inhibition via Dkk1. By comparing these conditions with controls, we observed that Wnt activation altered key inflammatory markers in glioma. The preliminary findings suggest that the Wnt/β-catenin pathway also modulates inflammatory reaction at the BBB in the CNS.

A6-3: Assessing the role of REG3β in inflammation-induced neuronal network formation during atherosclerosis

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Inflammatory reactions in arterial walls are a hallmark of atherosclerosis and contribute to the initiation and progression of the disease. Recently, neuroimmune cardiovascular interfaces (NICI) have been identified as important contributors to disease progression, but their underlying mechanisms remain unclear. Our previous studies identified REG3β as a key regulator of immune-inflammatory processes in acute cardiac remodelling. The present work aims to explore a potential involvement of Reg3 in settings of chronic cardiovascular disease (CVD), particularly atherosclerosis, being a well-known chronic inflammatory CVD causing ischemic heart disease, stroke, and peripheral vascular disease in humans. Current findings show that REG3β is downregulated in the aortic arch and serum of ApoE-/- mice at early atherogenesis. Reg3b-deficient mice in SMCs developed larger plaques. Further experiments of RNA –sequencing point out that the receptor Neuropilin-2 (Nrp2) and associated signalling molecules, including semaphorin 3 (Sema3) and Plexin A4 (Plxna4) were upregulated in leukocytes of ApoEsmcReg3b-/- mice, which have been associated with neuron development. In line with this, light sheet fluorescence microscopic analysis revealed an increased formation of Tuj+ neurons surrounding aorta of ApoEsmcReg3b-/- mice. Our present findings suggest that REG3β acts as a key regulator of neuroimmune crosstalk in atherosclerosis, where its loss promotes Nrp2-associated signalling, aberrant vascular innervation, and accelerated plaque progression.

A6-4: Lymphatic Neuromedin B Elevates Cardiac Damage Responses

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Intercellular cross talk between the cardiac microvasculature and other cardiac cells importantly regulates cardiac function in both health and disease. Important mediators of this cross-talk are angiocrine factors that are secreted by cardiac endothelial cells (EC). These factors have been implicated in heart development, homeostasis and damage responses. In order to find new angiocrine regulators that are relevant in the context of cardiac damage, we performed single-cell expression analysis after transverse aortic constriction (TAC), an experimental model for pressure-induced cardiac hypertrophy and heart failure. Our data suggest that neuromedin B (NmB) is significantly upregulated in lymphatic EC after TAC. However, the effect of NmB on cardiac and non-cardiac cells is still not studied. To address this, we tested the effect of the recombinant NmB in neonatal rat ventricular myocytes and neonatal cardiac fibroblasts. In neonatal rat ventricular myocytes, NmB facilitated endothelin-1 induced hypertrophy as indicated by upregulation of hypertrophy-specific genes such as Nppa, Nppb, Acta1 and increased cell size. In neonatal cardiac fibroblasts, NmB was shown to facilitate the fibrosis when given in combination with endothelin-1. In vivo, lymphatic EC (LEC)-specific deletion of NmB results in a cardioprotective phenotype when mice were subjected to TAC or an angiotensin-II pump infusion model. Postmortem analyses showed that LEC-specific NmB knockout animals displayed reduced cardiac hypertrophy and fibrosis. Mechanistically, we found that NmB facilitates Gq-mediated signaling to promote hypertrophy via the BB2/Grpr receptor. In the next steps, we will investigate the contribution of BB1/Nmbr receptor signaling and whether pharmacological inhibition of these receptor/s can reduce adverse cardiac remodeling.

A6-5: Lymphatic endothelial cell-derived neurotensin mitigates adverse cardiac remodelling

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Neuropeptide neurotensin is upregulated in cardiac lymphatic endothelial cells (LECs) in response to ischemic or mechanical damage, but its role in cardiac remodelling is unknown. Here, we aimed to define the role of LEC-derived neurotensin in cardiac injury responses and to assess the therapeutic potential of targeting cardiac NTS signalling. In vitro, neurotensin reduces cardiomyocyte hypertrophy and fibroblast activation, and these effects are mediated by neurotensin receptor 2 (NTSR2)-dependent cGMP production. LEC-specific deletion of neurotensin aggravates hypertrophy and fibrosis in mouse models of pressure overload and myocardial infarction, and the same is true for cardiomyocyte-specific or activated fibroblast-specific inactivation of NTSR2. NTSR2 agonist NT150 reproduces the beneficial effects of NTS in vitro and ameliorates cardiac remodelling and dysfunction in both myocardial infarction and pressure overload. Importantly, also in freshly isolated cardiac tissue from heart failure patients, NT150 induces cGMP production and suppresses pro-hypertrophic signalling. These findings identify NTS as an endogenous inhibitor of adverse cardiac remodelling and suggests NTSR2 agonists as a therapeutic strategy in heart failure.

A6-6: FLRT2 regulates blood-brain barrier repair after ischemic stroke

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Restoring blood-brain barrier (BBB) integrity after ischemic stroke is essential for limiting tissue damage and supporting functional recovery. Fibronectin leucine-rich transmembrane protein 2 (FLRT2) is a multifunctional adhesion and signaling molecule previously identified as a regulator of endothelial junction dynamics, vascular patterning, and BBB formation. Here, we investigated how FLRT2 contributes to vascular repair after stroke. Using the photothrombotic stroke model in adult mice, FLRT2 expression was found to increase strongly in perivascular fibroblasts within the ipsilateral lesion, suggesting an active role during post-stroke remodeling. Given the emerging role of perivascular fibroblasts in fibrotic scar formation and vascular stabilization after CNS injury, we next focused on whether fibroblast-derived FLRT2 contributes to BBB repair. To this aim, fibroblast-specific FLRT2-deficient mice were generated and subjected to photothrombotic stroke. Loss of FLRT2 in fibroblasts led to pronounced BBB disruption at 4 days after stroke, a critical phase associated with the onset of fibrotic scar formation and barrier repair. Mutant mice displayed macroscopic hemorrhages, corroborated by strong Ter119-positive erythrocyte extravasation, and enhanced astrogliosis, indicating aggravated vascular damage and an enhanced glial response. In addition, fibroblasts accumulated more prominently within the lesion core and peri-infarct region in FLRT2-deficient mice, suggesting a role for FLRT2 in fibroblast recruitment, organization, or retention at the injury site, potentially contributing to aberrant fibrotic repair. Collectively, these findings identify a cell type–specific role for fibroblast-derived FLRT2 as a critical regulator of BBB stability and fibrosis formation during the subacute phase of stroke.

A6-7: Trained Immunity in the Bone Marrow Links Metabolic and Uremic Stress to Cardiovascular Injury

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Chronic kidney disease (CKD) and metabolic disorders share an inflammatory phenotype that predisposes to cardiovascular events. We hypothesized that transient metabolic insults induce persistent, cell-intrinsic priming within hematopoietic stem and progenitor cells (HSPCs) in the bone marrow (BM), contributing to cardiovascular morbidity.

Using murine CKD and high-fat diet models, we quantified myeloid cells and mapped their spatial organization in the BM. Persistence and transferability were assessed via BM transplantation into naïve recipients. Functional consequences were evaluated following LAD coagulation-induced myocardial infarction (MI). Underlying mechanisms were explored using sorted HSCs and BM organoids. Clinical relevance was validated in three cohorts (>450,000 participants).

Kidney injury and HFD induced an expansion of myeloid progenitors and their relocalization towards BM sinusoids. This expansion persisted after resolution of kidney injury. Metabolic or uremic priming worsened post-MI cardiac function and augmented myeloid responses. CKD-trained BM transplanted into naïve recipients recapitulated this phenotype. Circulating ASC specks, released during inflammasome activation, were increased in murine serum and in patients with CKD or MI. Mechanistically, ASC specks induced myeloid expansion of long-term (LT)-HSCs in vitro. Multi-omics revealed ASC speck-induced metabolic and chromatin remodeling in LT-HSCs, leading to persistent myeloid bias. Transferring ASC speck-trained LT-HSC-derived cells into mice post-MI

worsened cardiac function. Clinically, higher plasma ASC specks correlated with increased cardiovascular risk in CKD.

Hematopoietic reprogramming represents a mechanism through which chronic inflammatory conditions and inflammasome activation contribute to long-term organ damage. Targeting drivers of hematopoietic memory, such as ASC specks, may offer novel strategies to prevent cardiovascular complications in CKD.

A6-8: FLRT2 regulates epithelial-mesenchymal transition to maintain the blood-retina barrier

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The blood–retina barrier (BRB) is a specialized barrier that regulates the movement of substances between the bloodstream and the retina, helping maintain a stable environment for proper visual function. The retinal pigment epithelial (RPE) cells are polarized cells that constitute the outer BRB by regulating the transport of nutrients and waste products to and from the retina. Age-related macular degeneration (AMD) is a retinal disease which is a leading cause of blindness and is characterized by degeneration of RPE cells, ultimately leading to outer BRB breakdown. In the exudative form of AMD, blood vessels from the choroid invade the retina resulting in choroidal neovascularization (CNV) and sub-retinal fibrosis. Epithelial-mesenchymal transition (EMT) has emerged as a defining feature in AMD, where the sub-retinal space is invaded by mesenchymal cells which are believed to originate from RPE cells undergoing EMT. The fibronectin and leucine-rich transmembrane protein 2 (Flrt2) is a membrane bound molecule that regulates functions in both nervous and vascular systems. We observed that Flrt2 is localized on the basal membrane of the RPE layer. Proximity ligation assay between Flrt2 and P-cadherin, which is the most dominant cadherin in RPE layer, reveals a proximity between the two proteins in the basal membrane of RPE layer. In-vivo deletion of Flrt2 in RPE cells (Flrt2idelRPE mice) resulted in altered baso-lateral polarity of P-cadherin and downregulation of Best1. We used laser induced CNV to model AMD in WT mice and observed that the RPE cells lose their polarity and Flrt2 changes its localization to apical as well as the basal membrane. Overall, our data indicates Flrt2 has a role in the maintenance of epithelial identity of RPE cells. We performed laser induced CNV in Flrt2idelRPE mice and observed that loss of Flrt2 in RPE cells aggravates the lesions at the laser injury site, suggesting Flrt2 has a protective role in the progression of AMD.

A6-9: Microenvironmental control of vascular barrier function in the CNS

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The blood-brain barrier (BBB) is an essential and complex structure, that mediates the functional interaction between the brain tissues and the circulatory system. It regulates the influx of necessary ions, enzymes, nutrient supply and the efflux of metabolic waste. At the same time, it maintains tight control over this exchange to support proper functioning of neurons and protect it from exposure to pathogens and toxins.

The WNT pathway is the main signaling cascades, inducing barrier function in brain endothelial cells. The canonical WNT pathway leads to activation of LEF1 and TCF Transcription factors via β-catenin signaling. TCF and LEF1 in turn activate multiple BBB-specific genes, such as glucose transporter 1 and Cldn5.

BBB present in almost all brain regions, except some circumventricular organs (CVOs) and the choroid plexus. In those areas, as well as at the transition zone on the brains surface, we can find a shift from non-leaky to leaky barrier phenotype. It is unknown how the barrier properties at such transition zones are organized and regulated, and we hypothesize it might be related to the WNT pathway activity in those areas. We performed multiple IF-staining on SFO whole mounts and CVO organ slices to track WNT activity in CVOs vasculature. The preliminary results show a gradual transition from BBB-specific phenotype to leaky phenotype alongside with the appearance of LEF1+ endothelial nuclei, suggesting a gradual regulation of the Wnt/β-catenin pathway. How this gradient of WNT activity is mechanistically achieved yet is to be discovered.

A6-10: Validation of Cardiovascular Magnetic Resonance Against Invasive Haemodynamics in Patients with Heart Failure with Preserved Ejection Fraction (DECIPHER HFpEF DZHK-12)

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Heart failure with preserved ejection fraction (HFpEF) is heterogeneous and difficult to diagnose noninvasively. Invasive pressure–volume (PV) loops are the reference standard for diastolic mechanics but impractical for routine use. Cardiovascular magnetic resonance (CMR) can assess fibrosis, inflammation, and microvascular dysfunction, but multicenter validation against invasive haemodynamics is lacking.

Objectives: To validate CMR tissue characterisation and perfusion against invasive PV-loop indices (TAU, BETA) in HFpEF, and test whether CMR improves prediction beyond echocardiography and NT-proBNP.

Methods: DECIPHER-HFpEF-DZHK-12 (NCT03251183) is a prospective, multicenter study across 7 German academic hospitals. Symptomatic patients (NYHA II–III, LVEF >45%) with elevated filling pressures underwent CMR and invasive PV-loops alongside age-gender-matched controls and healthy volunteers. Core CMR parameters: native T1, T2, and microvascular disease (MVD) duration. Multivariable regression compared a base model (log NT-proBNP, E/e', LAVI, LVMi) versus base plus CMR. A subset underwent endomyocardial biopsy.

Results: 113 HFpEF patients (97 with evaluable PV loops), 33 matched controls, 18 healthy volunteers. T1, T2, and MVD duration were elevated in HFpEF versus both control groups (p<0.001). A combined CMR model achieved cross-validated AUC 0.93. TAU was independently associated with T2, E/e', and LV-EDVi; BETA with T1, T2, NT-proBNP, and LV-EDVi. Adding CMR significantly improved prediction of TAU and BETA

(p<0.03 and p<0.0001). Biopsy (n=70) showed inflammation in 18.6% and fibrosis in 44%; collagen metrics correlated with BETA and T1.

Conclusions: CMR markers of fibrosis, inflammation, and microvascular dysfunction differentiated HFpEF from controls and improved noninvasive prediction of invasive PV-loop indices beyond echocardiography and NT-proBNP, supporting CMR incorporation into HFpEF diagnostic pathways.

A6-11: Spatial Cell–Cell Communication in Alzheimer's Disease under sEH-KO

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Amyloid precursor protein (APP) is a transmembrane protein expressed in neurons and is involved in synapse formation and cell signaling. In the amyloidogenic pathway associated with Alzheimer's disease, APP contributes to the generation of amyloid-β (Aβ) peptides. These peptides aggregate into plaques, disrupt synaptic function, activate astrocytes, and induce metabolic dysfunction.

In Alzheimer's disease, astrocytes involved in Aβ clearance become reactive, leading to reduced clearance capacity and a shift toward a pro-inflammatory state. Epoxyeicosatrienoic acids (EETs) counteract this by reducing astrocyte reactivity. However, soluble epoxide hydrolase (sEH), which degrades EETs, is upregulated near plaques. Genetic deletion of sEH (sEH-KO) is therefore associated with decreased astrocyte reactivity and improved metabolic function.

We analyze 10x Genomics Visium spatial transcriptomics data at spot resolution from APPF1 mouse brain tissue samples, comparing sEH-KO and control conditions. Our analysis focuses on spatially resolved gene expression changes associated with astrocyte signatures and metabolic pathways, investigating how sEH-KO influences astrocyte metabolism.

Prior studies have addressed the effects of sEH-KO as a modulator of Alzheimer's pathology, but less is known about how these effects are spatially organized within brain tissue. By applying spatially resolved transcriptomic analysis, we investigate the topological structure of cell–cell communication networks associated with astrocyte transcriptomic and metabolic shifts.

Our analysis pipeline includes quality control, cell type deconvolution, and spatially aware clustering. We further explore cell-cell communication to identify ligand-receptor (LR) interactions between astrocyte and neighboring cell types. Further, we investigate LR mediated communication patterns and localize functional hotspots within the brain.

A6-12: Neurovascular Mechanisms Driving Dynamic Spatiotemporal Remodeling of Cardiac Innervation After Myocardial Infarction

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Cardiovascular diseases are the leading cause of death, with myocardial infarction (MI) being highly prevalent. Unlike adult mammals, zebrafish and neonatal mice can regenerate their hearts after injury, a process influenced by the autonomic nervous system (ANS). However, the role of ANS in adult cardiac repair remains unclear. Here, we investigated ANS remodelling after MI and its molecular regulation.

Histological profiling of mouse hearts 3-28 days after MI revealed a transient loss of sympathetic innervation in the infarct and border zones, peaking at day 14 (up to 22-fold decrease vs. control), followed by partial re-innervation at day 28. The remote zone showed stable or slightly increased innervation. Single-cell RNA sequencing indicated dynamic regulation of axon guidance cues, particularly in vascular cells: neuroprotective factors (Ntf3, Vegfb) decreased acutely and recovered later, while repulsive cues (Sema3a, Flrt2) showed the inverse pattern. Flrt2 expression peaked at day 7 in infarct regions and decreased thereafter. Spatial transcriptomics confirmed its localization to the injured zone. Endothelial-specific Flrt2 deletion enhanced cardiac innervation in the atria, implicating Flrt2 in post-MI neural remodelling.

Our data suggest MI induces transient denervation followed by re- and hyperinnervation mediated by endothelial axon guidance signals, offering potential therapeutic targets for post-MI remodelling.

A6-13: A Novel Endocardial–Axon Axis Links Aging to Left Atrial Hyperinnervation and Atrial Fibrillation Risk

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With increasing life expectancy, age-related cardiovascular diseases have become a leading cause of mortality. Cardiac aging induces structural and functional changes that predispose to arrhythmias. While endothelial cell (EC) senescence causes left ventricular (LV) denervation, the impact of aging on atrial innervation remains unclear. Given the role of autonomic remodeling in atrial fibrillation (AF), we investigated innervation patterns and neurovascular cross-talk in aging atria.

Tuj1-staining revealed significant differences between 3-month-old mice and 22-month-old mice, with a 1.6±0.1-fold increase (p=0.01) in left atrial (LA) innervation. LA hyperinnervation was driven by increased sympathetic fibers, confirmed by TH-staining (1.6±0.1-fold; p=0.0003). Single-nuclei RNA sequencing of young and old mouse LAs showed downregulation of axon-repelling factors specifically in endocardial ECs, including Flrt2. FLRT2 exhibited strong axon-repellent activity in sympathetic neuron cultures, and EC-specific Flrt2 deletion increased LA innervation (1.3±0.1-fold; p=0.07).

Supernatant from aged sympathetic neurons increased the beating frequency of iPSC-derived atrial cardiomyocytes compared to young neurons

As this cross-talk might contribute to arrhythmias, we studied hearts from young and old mice for AF susceptibility in vivo. Electrical burst pacing revealed that AF burden correlates with LA hyperinnervation in aged mice, which have a higher susceptibility (p=0.07) for induced AF than young mice. Clinical relevance was provided by histological analysis of human LA samples that confirmed 1.3±0.1-fold (p=0.09) higher innervation levels in AF patients compared to non-AF patients.

In conclusion, we propose a novel endocardial-axon-axis driving hyperinnervation in the aging LA, which may contribute to AF. Targeting EC-derived neuronal guidance cues may represent a new therapeutic approach to prevent age-related AF.

A6-14: Metabolic alterations drive inflammatory phenotypes in CHIP-associated heart failure

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Mutations in DNA methyltransferase 3 alpha (DNMT3A) are the most frequent driver of clonal hematopoiesis of indeterminate potential (CHIP), and associated with higher risk of cardiovascular disease and pro-inflammatory activation of immune cells. Here, we investigated the mechanisms underlying DNMT3A CHIP-associated inflammatory phenotypes in macrophages. We analyzed DNA methylation and metabolic profiles in patients with heart failure (HF) and chronic obstructive pulmonary disease (COPD), comparing individuals with and without DNMT3A CHIP mutations. Patients with DNMT3A-mutant CHIP exhibited a globally hypomethylated DNA profile alongside elevated levels of tricarboxylic acid (TCA) cycle metabolites, particularly malate and fumarate. Mechanistically, we found that DNMT3A mutations are associated with hypomethylation of the succinate dehydrogenase A (SDHA) gene results in a rewired TCA cycle and enhanced activity of mitochondrial complex II in macrophages. Consistently, DNMT3A knockdown in macrophages phenocopied the CHIP-associated mutation, increasing SDHA mRNA levels and complex II activity. We further identified

malate, a metabolite linked to complex II activity, as a key mediator of inflammation. Malate is released extracellularly, crosses cell membranes, and re-enters the TCA cycle in recipient cells, thereby promoting inflammatory activation in macrophages and amplifying inflammation. Spatial metabolomics analysis revealed increased malate levels and elevated SDHA activity in the hearts of DNMT3A-mutant mice. Importantly, pharmacological inhibition of SDHA using dimethyl malonate improved inflammatory responses and cardiac function following myocardial infarction. Collectively, these findings identify a DNMT3A–SDHA–malate axis that links epigenetic dysregulation to metabolic reprogramming and inflammation. Targeting metabolic alterations may represent a promising therapeutic strategy to attenuate inflammatory activation in patients with DNMT3A-driven CHIP.

A6-15: Epicardial Adipose Tissue Inflammation Drives Right Ventricular Failure in PAH

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Background: Right ventricular (RV) dysfunction is the strongest predictor of morbidity and mortality in pulmonary arterial hypertension (PAH), yet the mechanisms driving RV failure remain poorly understood. Epicardial adipose tissue (EAT), a metabolically active fat depot implicated in left heart failure, has not been explored in RV dysfunction. Objective: To investigate the contribution of EAT to RV dysfunction in PAH using an integrated multi-omics and functional approach. Methods and Results: EAT samples from the RV of PAH patients with decompensated RV function (n=8) and age-matched controls (n=8) were analyzed by transcriptomic and proteomic profiling. PAH-EAT showed major molecular alterations with enrichment of inflammatory signaling and extracellular vesicle (EV) pathways. BATLAS deconvolution and gene ontology analyses revealed a shift toward white pro-inflammatory adipocytes, supported by hypertrophic PLIN1^{+} adipocytes with reduced UCP1, increased M1 macrophages (CD68^{+}CD80^{+}), and B-cell activation (CD45^{+}CD19^{+}). Single-nucleus RNA sequencing confirmed immune cell enrichment, particularly B cells. Functionally, conditioned media (CM) from PAH-EAT induced RV fibrosis, cardiomyocyte hypertrophy and apoptosis ex vivo (human RV slices), promoted fibroblast migration and macrophage polarization, and increased cardiomyocyte death in vitro. Free cytokine levels in CM were unchanged, whereas EV lysis revealed elevated pro-inflammatory mediators, notably IL-17. Recombinant IL-17 reproduced the deleterious effects of PAH-EAT CM ex vivo. Conclusion: PAH-EAT displays a pro-inflammatory phenotype that contributes to RV dysfunction. EAT-derived EVs emerge as key paracrine mediators of RV injury, revealing a novel mechanistic link between adipose tissue biology and RV failure in PAH.

A6-16: Fibronectin-Integrin α5β1 Axis as a Determinant of Right Ventricular Failure in Pulmonary Arterial Hypertension

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Introduction: Right ventricular (RV) function is a key prognostic factor in pulmonary arterial hypertension (PAH). In response to pressure overload, the RV undergoes remodeling driven by extracellular matrix (ECM) accumulation and cardiomyocyte hypertrophy. Integrins are cell adhesion receptors that transduce extracellular cues through ECM binding, activating pro-hypertrophic and pro-fibrotic pathways. The fibronectin-binding integrin α5β1 is one of the most abundant heterodimers in cardiac tissue. We hypothesized that integrins represent a potential therapeutic target for RV dysfunction in PAH.

Methods and results: Using publicly available transcriptomic datasets, we identified the "ECM-receptor interaction" pathway as enriched in RVs from PAH patients and animal models. Among its components, ITGA5 and fibronectin were consistently upregulated. Western blot confirmed increased α5 and β1 levels in PAH RVs, correlating with disease severity. In rat cardiomyocytes, α5β1 inhibition reduced phenylephrine-induced hypertrophy, reversed established MCT-induced hypertrophy (F-actin labeling), and enhanced contractility (sarcomere shortening, time to peak). In human RV fibroblasts, α5β1 inhibition prevented TGFβ1-induced activation and decreased proliferation and activation of PAH-derived cells (PCNA, αSMA, COL1; Ki67). In pulmonary artery banding and monocrotaline rats, α5β1 inhibition improved RV function (CO, TAPSE, ejection fraction, strain; echocardiography, RHC, MRI) and reduced RV hypertrophy and fibrosis. Ex vivo, α5β1 inhibition attenuated hypertrophy and fibrosis and enhanced contractile work in human precision-cut RV slices.

Conclusion: Integrin α5β1 plays a central role in RV dysfunction in PAH. Phosphoproteomic studies are ongoing to further define the underlying mechanisms

A6-17: Unmasking right ventricular remodeling: From RHF core gene signature to specialized cardiac niches

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Heart failure (HF) remains the leading cause of premature death worldwide and is characterized by the progressive inability of the heart to pump sufficient blood volume to the body's organs. Current treatment strategies differ in their efficacy in left- vs. right-sided HF, reflecting the distinct embryonal origin, anatomy, and biomechanical environment of the right ventricle (RV). To uncover the underlying molecular basis of RV-specific pathological remodeling, we employed a multi omics framework in both rat pulmonary artery banding (PAB) models and samples from human patients. Multi-level bioinformatic analyses from 388 RNA-seq and proteomics data sets were integrated to identify genetic networks specific to ventricles, disease states, and the type of heart failure. With this systematic –omics approach we identified a RHF core signature of 113 genes specific for the hypertrophic and failing RV.

Subsequently, genome-wide spatial transcriptomics analyses allowed us to resolve these altered genetic networks with high spatial resolution in rat whole heart tissue sections. In addition to localized regulation of RHF core signature genes, the results suggested the existence of cardiac niches comprising activated cells within distinct heart regions. Single molecule RNA FISH analyses with probes against marker genes of specialized cardiomyocytes and fibroblasts validated these observations at the single cell level and now serve as a basis for further characterization of RHF cardiac niches.

Besides a further systematic evaluation of smRNA FISH data across rat PAB models, we plan to investigate RHF cardiac niches at the proteomic level as well as to perform meta-analyses with other spatial transcriptomics and single cell datasets. In the long-term, these analyses will form the basis to modulate genetic networks, rather than individual genes in RHF by cardiomyocyte specific knockouts and AAV dCas9 KRAB mediated gene silencing.

A6-18: SOX9-Dependent Transcriptional Switch Controls Endothelial Plasticity in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a progressive, fatal vascular disease with limited curative options. Endothelial-to-mesenchymal transition (EndoMT) has recently emerged as a key mechanism driving vascular remodeling in PAH (Lu et al., 2019), a process normally active during development and tissue repair. Previous work from our group (Chelladurai et al., 2022) showed that several development-associated transcription factors, including SOX9 and TBX4, are activated under pathological conditions. This study investigates the role of SOX9 in disease progression.

Functional studies initially demonstrated that targeting SOX9 alone in pulmonary artery endothelial cells (PAECs) and fibroblasts from idiopathic PAH patients partially rescued the disease phenotype. In murine precision-cut lung slices (PCLS), Cre-induced knockout of SOX9 in mesenchymal cells attenuated vascular remodeling triggered by a predefined growth factor cocktail. Single-cell dataset analysis and PCLS staining revealed a distinct cell population in an intermediate EndoMT state, characterized by high SOX9 expression. Upon disease induction, this unique population transitions more rapidly to a mesenchymal-like state, initiating vessel remodeling.

Transcriptomic analysis combined with siRNA-mediated silencing of SOX9 showed that SOX9 acts as a pioneering factor, inducing the mesenchymal program while repressing the endothelial signature, primarily by regulating TBX4 and SOX17 expression.

Building on these findings, we hypothesize that SOX9 overexpression drives EndoMT in PAECs by disrupting the TBX4–SOX17 balance. Our in vitro studies confirm that SOX9 overexpression alone is sufficient to reprogram PAECs to a mesenchymal phenotype. By defining the SOX9–TBX4–SOX17 transcriptional circuit, this work provides a mechanistic framework for future therapeutic interventions in PAH.

A6-19: Role of TGFβ-modulated long non-coding RNA, VIM-AS1, in Pulmonary Hypertension

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It is known that an imbalance in TGFβ and BMP signaling plays a crucial role in the pathogenesis of pulmonary hypertension (PH). The role of TGFβ signaling in modulating lncRNAs is less explored in PH. The main aim of this study is to characterize the role of the TGFβ-regulated lncRNA vimentin antisense 1 (VIM-AS1) in PH. VIM-AS1 expression is significantly upregulated in PASMCs from idiopathic PAH patients and in the lungs of patients diagnosed with group 3 PH (COPD-PH and IPF-PH). GapmeR-based silencing of VIM-AS1 resulted in anti-proliferative and pro-apoptotic effects. Furthermore, treatment of IPAH-PASMCs with sotatercept (an activin/TGFβ ligand trap) reduced VIM-AS1 expression and induced anti-proliferative and pro-apoptotic effects. To delineate the molecular mechanism mediated by VIM-AS1 in PH, the localization of the lncRNA was investigated and found to be exclusive to the nucleus, suggesting a role in transcriptional or epigenetic regulation. Previous studies have shown that VIM-AS1 can form RNA-DNA loops that alter DNA methylation in promoter regions. Interestingly, [3]24 differentially expressed genes (DEGs) were found to be enriched for RNA-DNA loop-forming regions in IPAH PASMCs, indicating the importance of this lesser-known mechanism in PH. In silico screening for RNA:DNA:DNA triplex-forming regions revealed that 113 regions on DNA can interact with VIM-AS1 transcripts. Specifically, we found that 3,[7]00 genes were altered by VIM-AS1 silencing, of which 104 genes had altered DNA methylation patterns in IPAH PASMCs. Pathway analysis showed that the TGFβ signaling pathway was strongly affected by knockdown of VIM-AS1. R-loop formation mediated by VIM-AS1 and its role in modulating chromatin accessibility of TGFβ signaling genes is being investigated using DRIP-Seq. Further, we will investigate the therapeutic potential of VIM-AS1 by targeting the rat ortholog of VIM-AS1 in the MCT and Sugen-hypoxia models of PH and in human precision-cut lung slices.

A6-20: Endothelial AK4 regulates pulmonary vascular remodeling in hypoxia-induced pulmonary hypertension and exhibits sex-dependent effects

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Pulmonary hypertension (PH) is an incurable disease with a higher prevalence in females. Pathological vascular remodeling is a hallmark of PH, characterized by excessive muscularization of distal pulmonary vessels, and loss of the pulmonary capillary network. Previously, we showed that adenylate kinase 4 (AK4) promotes proliferation in pulmonary artery smooth muscle cells (PASMC) under hypoxic conditions and is increased in the intimal and medial layers of remodeled vessels in PH patients. However, the role of AK4 in endothelial cells (EC) during PH pathogenesis remains unclear.

To address this, AK4 was silenced in vitro using small interfering RNA (siRNA) in EC exposed to normoxia (21% O_2) or hypoxia (1% O_2). Angiogenic behavior was assessed by 3-dimensional (3D) tube formation assay, and EC-PASMC co-culture system was used to investigate the impact of silencing AK4 in EC on PASMC. For in vivo studies, endothelial-specific Ak4 knockout mice (Tie2CreERT2; floxed AK4) were exposed to 21% O_2 or 10% O_2. PH severity was measured by right heart catheterization, and the degree of pulmonary vascular remodeling was assessed by histological analyses.

Under hypoxia, AK4 expression was upregulated in EC. AK4 loss-of-function increased tube numbers with smaller diameters. In the co-culture system, AK4 silencing in EC suppressed PASMC proliferation. Importantly, Tie2+-specific Ak4 knockout protected only female mice from hypoxia-induced PH, as indicated by lower right ventricular systolic pressure and reduced vascular pruning.

These findings identify endothelial AK4 as a regulator of hypoxia-driven pulmonary vascular remodeling with a female-specific protective effect upon deletion. Future studies will investigate the effect of endothelial AK4 on neo-muscularization of distal pulmonary vessels and try to identify the sex-dependent mechanism that drives the different response between male and female mice.

A6-21: Maternal and perinatal obesity disrupt neuro-autonomic cardiac interface across generations

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Conclusion: Together, these findings identify a mechanistic link between maternal and perinatal obesity and aberrant neuro-ANS interface across generations, possibly underlying priming of susceptibility to CVD.

A6-22: Cytochrome c Oxidase Subunit 4 Isoform 2 (COX4I2) Mediates Acute Hypoxia-induced Reduction of Mitochondrial Electron Transport System in Pulmonary Artery Smooth Muscle Cells

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Pulmonary arterial smooth muscle cells (PASMC) trigger hypoxic pulmonary vasoconstriction (HPV) by sensing acute hypoxia (AH), matching perfusion to ventilation to avoid hypoxemia. The cytochrome c oxidase subunit 4 isoform 2 (COX4I2) is essential for sensing AH because it spikes mitochondrial superoxide release, promoting HPV. However, its upstream molecular signal remains unclear. We hypothesised that COX4I2 influences the redox state (RedOx) of the electron transport system (ETS) during AH.

Pulmonary arterial pressure (PAP) was measured in isolated, perfused and ventilated lungs from wild type (WT) and Cox4i2-/- (KO) mice exposed to AH, anoxia, and KCN. NAD+ and coenzyme Q (CoQ) RedOx were analysed simultaneously with oxygen consumption in isolated PASMC, using a NextGen-O2K oxygraph and RAMAN spectroscopy. Complexome profiling was investigated by mass spectrometry. The relevance of COX4I2-specific cysteine residues for AH-induced superoxide release was tested by analysing superoxide production and oxygen affinity in CMT167 expressing either WT or mutant COX4I2.

The increase of PAP induced by AH and KCN in WT was blunted in KO mouse lungs. On the contrary, anoxia decreased PAP in the WT and increased it in KO. Hypoxia-induced reduction of NAD+, CoQ, and cytochrome c was diminished in KO PASMC. Oxygen consumption, COX assembly and supercomplex formation were similar in WT and KO PASMC, with COX4I2 peptides detected only in the monomeric COX. CMT167 cells expressing either COX4I1 or COX4I2 mutants lacked hypoxia-induced superoxide release, which was detected only in cells expressing WT COX4I2.

Our findings demonstrate that COX4i2 favours the ETS reduction induced by AH, promoting superoxide release.

HUB 1

H1-1: Obesity induced endothelial-to-mesenchymal transition involves PKA-mediated TGF-β receptor activation

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Obesity has recently been shown to promote partial endothelial-to-mesenchymal transition (EndMT), which may contribute to obesity-associated insulin resistance. Although EndMT is commonly driven by transforming growth factor-β (TGF-β) receptor signaling, whether obesity-induced EndMT involves TGF- β signaling pathway and how it is activated in this context remains unclear. Here, we identified the transmembrane protein TMEM120A as a scaffold protein which interacts with PKA and AKAP9 at the Golgi apparatus. Loss of endothelial TMEM120A resulted in PKA activation and EndMT in vitro and in vivo and increased obesity-induced EndMT. We found that PKA can directly phosphorylate and activate TGF-□-receptor 1 (TGFBR1) in a ligand-independent manner. Genetic ablation of endothelial Gαs/PKA signaling or of endothelial TGFBR1 attenuated obesity-induced EndMT and improved glucose tolerance in obese mice whereas endothelial loss of TMEM120A reduced glucose tolerance. This suggests a central role for the endothelial PKA–TGFBR1 axis in linking obesity to EndMT-driven metabolic dysfunction and highlights a potential therapeutic target for obesity-associated type 2 diabetes.

H1-2: Repair and Modular Turnover Dynamics of Mitochondrial Complex I: Differential Responses to Loss of the High-Turnover Subunits NDUFA6 and NDUFA7

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Mitochondrial respiratory complexes maintain functionality through selective turnover and replacement of damaged subunits. The mechanisms coordinating damage recognition, targeted extraction, and subunit replacement remain poorly understood. In complex I, turnover preferentially targets peripheral N and Q modules via ClpXP-mediated removal and replacement of subunits. High-turnover components, including NDUFA6 and NDUFA7, are late-incorporating and weakly integrated, with the cochaperone DNAJC30 facilitating their exchange.

Here, we investigated the role of the high-turnover subunits NDUFA6 and NDUFA7 in complex I stability and repair by generating cellular knockout models designed to locally destabilize the N module while preserving partial enzymatic activity. Complex I–dependent respiration was reduced in both models (38–65%), confirming functional impairment despite residual activity. Quantitative complexome profiling revealed distinct effects on complex I organization. Loss of NDUFA6 caused pronounced destabilization of the N module accompanied by a global reduction of other modules, consistent with structural collapse of the complex. In contrast, NDUFA7 deficiency resulted in a more homogeneous decrease in abundance across all modules (~25%). Both mutations triggered upregulation of mitochondrial quality control and service factors, including DNAJC30 and the chaperone DNAJA3, indicating activation of repair pathways. These findings suggest distinct cellular responses: a “demolition and rebuild” strategy following structural collapse in NDUFA6 KO cells, and a more controlled maintenance response in NDUFA7 KO cells. Ongoing pulse SILAC experiments combined with mitochondrial stress aim to resolve subunit-specific turnover kinetics and further dissect the balance between damage accumulation and repair. Together, our results provide evidence for distinct, subunit-dependent repair strategies in complex I and highlight modular turnover.

H1-3: Personalized Detection of Left Ventricular Hypertrophy Using Model-Based Prediction Intervals: Comparison with Standard Cutoffs and UK Biobank Reference

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Background: Left ventricular hypertrophy (LVH) on cardiovascular magnetic resonance (CMR) is conventionally defined by fixed sex-specific LV mass index (LVMi) cutoffs (>75 g/m2 male, >59 g/m2 female). These ignore modifiable risk factors that physiologically influence LVM. We compared personalized model-based LVH detection against standard cutoffs and externally derived UK Biobank (UKB) coefficients.

Methods: From a prospective CMR registry (n=2,235; 54% female; median age 48), we built two regression models for LVMi: a full model (BMI, SBP, DBP, smoking, diabetes, exercise [MET-min/week], sex; R2=0.30) and a simplified model (sex, SBP, BMI, smoking; R2=0.29). Subject-specific upper 95% prediction intervals served as personalized LVH cutoffs. A fourth model applied published UKB multiplicative coefficients (Petersen 2017) to our cohort. All four approaches (standard reference, full, simplified, UKB) were evaluated against clinically annotated LVH (n=52 positive) using ROC analysis and confusion matrices.

Results: Personalized models substantially outperformed standard cutoffs: AUC 0.969 (full), 0.968 (simplified), and 0.956 (UKB) vs 0.886 (reference). The simplified 4-variable model performed comparably to the full 7-variable model. At the binary classification threshold, the reference cutoff achieved 78.8% sensitivity and 82.9% specificity; the personalized models achieved higher specificity (98.0%) at the cost of sensitivity (63.5%); the UKB model showed near-perfect sensitivity (98.1%) but lower specificity (52.5%). A 4-variable model was sufficient to improve LVH discrimination over conventional cutoffs.

Conclusion: Personalized prediction-interval-based LVH cutoffs markedly improve discrimination over standard references. A parsimonious 4-variable model performs equivalently to a full model. External UKB coefficients transfer well to clinical CMR populations, confirming generalizability of population-derived LVM determinants.

H1-4: Allometric Normalization and Z-Score Reference Values for Left and Right Atrial Area from Cardiovascular Magnetic Resonance

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Background: Atrial size is a key marker of diastolic dysfunction, valvular disease, and arrhythmia risk. Current indexing approaches (BSA, height) incompletely remove body-size dependence, particularly across the BMI spectrum. We derived allometric normalization models and z-scores for left (LAA) and right atrial area (RAA) from cardiovascular magnetic resonance (CMR) in a clinical cohort with a defined healthy reference subgroup.

Methods: From 1,430 subjects in a prospective CMR registry, we defined a reference cohort (superhealthy n=15, healthy n=588) free from hypertension, diabetes, and high-level athletic training. Log-linear allometric regression estimated height and BMI exponents for LAA and RAA, adjusting for age (natural splines, 4 df) and sex. Z-scores were computed as standardized residuals from the reference model and applied to all subjects including 651 disease and 176 CMR-normal patients. Residual correlations with height and BMI were compared across indexing methods (BSA-indexed, height-allometric, height-BMI-allometric).

Results: Estimated allometric exponents were LAA ~ height^1.85 x BMI^0.32 and RAA ~ height^1.88 x BMI^0.33 (all p2 SD) compared to 2.6% in the healthy reference. RAA z-scores showed similar discrimination. Disease patients showed wider z-score dispersion than healthy subjects.

Conclusion: Allometric normalization using data-derived height and BMI exponents provides body-size-independent atrial area indices superior to conventional BSA indexing. The resulting z-scores enable clinically intuitive identification of abnormal atrial enlargement in individual patients, applicable across the BMI spectrum.

H1-5: Automated Classification of Cardiovascular Magnetic Resonance Reports Using Large Language Models and Unsupervised Clustering

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Background: Clinical cardiovascular magnetic resonance (CMR) reports contain valuable diagnostic information, but manual interpretation is time-consuming and prone to inconsistencies. We developed an automated pipeline combining large language models (LLMs) with unsupervised clustering to extract and phenotype cardiovascular diagnoses from free-text reports.

Methods: We processed [1,2]72 unstructured CMR reports using the Stanza NLP library (tokenization, lemmatization, named entity recognition) and the OpenAI gpt-3.5-turbo API in a three-stage workflow: Extraction of confirmed and rejected diagnoses, Categorization into higher-level cardiological categories, and Quantitative Labelling. K-means clustering (k=5, elbow method) was applied to ten CMR parameters (T1, T2, LVEF, EDVi, LVMi, RVEF, LAA, LGE_no, LGE_ischemic, LGE_nonischemic). Clusters were compared against original clinical diagnoses (normal, IHD, CMP, HF, myocarditis). Cases in unexpected clusters were analysed for misclassification sources.

Results: Misclassifications were primarily attributed to missing clinical categories in model prompts, followed by human database errors and LLM limitations. Iterative refinement expanded the scheme to 12 diagnostic categories including HFpEF, myocarditis with and without myocardial damage, and diffuse cardiac involvement. CMP and HF showed no cluster specificity due to phenotypic heterogeneity. HFpEF and myocarditis without damage occupied two clusters, associating either with normal phenotype or with diffuse myocardial damage and inflammation, the latter shared with CMP and inflammatory conditions.

Conclusion: LLM-based NLP combined with unsupervised clustering provides a scalable approach to CMR report classification, identifying and reducing human error while enabling nuanced diagnostic categorization. The pairing of a diagnostic category with a phenotype cluster provides intuitive patient characterization.

H1-6: A Proteomics-Enhanced Machine Learning Framework for Predicting Incident Aortic Valve Disease and Identifying Functionally Relevant Proteins

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Aortic valve disease (AVD) is frequently diagnosed at advanced stages due to the absence of sensitive early biomarkers, and traditional clinical risk factors provide limited predictive accuracy. This study evaluated whether large-scale plasma proteomics could improve prediction of incident AVD and identify biologically relevant proteins. We conducted a prospective analysis in the UK Biobank, including participants with baseline clinical data and ~3,000 Olink plasma proteins. Individuals free of AVD at baseline were followed for incident events. The cohort was split into a 2/3 training set and a 1/3 independent validation set. Proteome-wide association analyses were performed using Cox proportional hazards models with Bonferroni correction, identifying 68 proteins significantly associated with incident AVD, which were consistently replicated. A multi-step feature selection pipeline was then applied, including information gain ranking and sequential forward selection to derive an optimal protein signature. An XGBoost model integrating clinical variables and selected proteins was trained using cross-validation and evaluated in the independent validation set. Functional validation was performed using candidate proteins selected from top-ranked features. Experiments in fibroblasts and valvular interstitial cells (VICs) assessed calcification and osteogenic differentiation. Proteomic data improved prediction of incident AVD beyond clinical models. The integrated model achieved AUCs of 0.821 for 5-year and 0.792 for >5-year prediction, outperforming demographic-only models (AUC 0.695 and 0.732, respectively), with consistent performance across the full follow-up (AUC 0.801 vs 0.730). A compact protein signature showed stable predictive performance. Among top-ranked proteins, IGFBP7 and CXCL17 were consistently associated with incident AVD. IGFBP7 is linked to cellular senescence, fibrosis, and has also been implicated in calcification-related processes, while CXCL17 mediat

H1-7: Discovery of disease genes by combining transcription factor, epigenome and DNA variation data

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Genome wide association studies (GWAS) identified thousands of common but also rare genetic variants, such as Single Nucleotide Variants (SNVs) associated to traits or diseases. A substantial amount of these variants is non-coding and might be located within regulatory elements. Thus, they can affect Transcription Factor (TF) binding sites and therefore alter the target gene expression. Identifying those genes is a crucial step in understanding the moleculare mechanisms underlying a trait or disease.

Previously developed method for disease gene identification often include DNA variation and/or epigenome data, however the regulatory aspect is often omitted. Especially for non-coding SNVs considering altering TF binding is important to understand the regualtory mechanisms of disease genes in more detail.

In this work, we present an approach for the interpretation of rare and common non-coding variants, by identifying regulatory SNVs (rSNVs), which are predicted to affect transcription factor binding sides. Furthermore, we integrate epigenome data to link the rSNVs to target genes and apply different statistical methods to aggregate information of TFs and genes. To prioritise disease genes with high confidence, we model for each gene the expected number of CREs overlapping with rSNVs using the Poisson-binomial distribution, taking ito account the LD structure of the analysed SNVs. Using GWAS from different complex diseases, we showcase that our approach can identify disease relevant genes, whether protein-coding or non-coding RNA and also highlight relevant genes not found with a well-established method. In addition, we illustrate the usefulness of our approach using epigenome and variation data from the epiATLAS, for instance to predict disease-specific losses or gains of TF binding sites across neuropsychiatric disorders.

H1-8: An Analysis Pipeline for Subcellular Spatial Transcriptomics in an ACLF Mouse Model: Dataset and Challenges

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The liver is the largest solid organ in the human body and plays a central role in the bodies immune response. Acute on-Chronic Liver Failure (ACLF) describes a form of acute decompensation of the liver, characterized by the failure of at least one of six major organ systems (liver, kidney, brain, coagulation, circulation, and respiration). The condition carries a devastating 28-day mortality rate of 30-40%, for which, to date, no effective therapeutic strategies exist. This is of particular concern given the rising prevalence of chronic liver disease driven by Western dietary patterns, making a deeper understanding of ACLF progression and underlying mechanisms increasingly urgent.

To address this need, we present a spatially resolved mouse liver dataset spanning the ACLF disease progression. Tissue sections from 12 male mice were processed across two independent experiments using the spatial transcriptomics platform CosMx™ SMI (NanoString), yielding gene expression profiles for nearly 1,000 selected markers at subcellular resolution. Here we describe a computational pipeline for processing this dataset, while also transparently highlighting unresolved analytical challenges that may serve as a basis for future methodological development.

H1-9: Establishment of an aged bronchioalveolar lung organoid (BALO) model to study epithelial-mesenchymal crosstalk during influenza virus (IAV) infection

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Age is a major risk factor for influenza A virus (IAV) infection-associated morbidity and mortality. The mechanisms by which aging impairs the antiviral response remain poorly understood, highlighting the need to develop advanced systems that recapitulate the lung microenvironment, support viral infection, and enable intercellular crosstalk investigation. Pharmacological screening in murine alveolar epithelial cell (AEC) monolayers identified D-galactose (D-gal), hydrogen peroxide (H2O2), doxorubicin, and the NAD+ biosynthesis inhibitor daporinad (FK866) as potent inducers of cellular senescence marked by a transcriptional upregulation of senescence (p21, p16, Trp53) and proinflammatory (IL-1β, IL-6) genes. Unbiased transcriptomic analysis of 84 aging-associated genes confirmed that D-gal-treated AECs adopt a pro-senescent transcriptomic profile, closely mimicking that of AECs isolated from naturally aged mice. Similarly, in D-gal-treated rMCs, an overall senescent profile was confirmed. Notably, a significantly shortened telomere length and reduced levels of NAD+ were observed in D-gal-treated AECs. Building on this data, the hallmarks of aging were systematically investigated in a 3D bronchoalveolar lung organoid (BALO) model. D-gal BALO exposure significantly increased senescence marker expression and DNA damage and reduced overall organoid-forming efficiency (OFE), while FK866 treatment caused DNA damage without affecting OFE. Introduction of stiff matrix, a key feature of aging lungs, markedly impaired organoid formation and induced senescence without any additional treatment. D-gal/FK866 treatment and IAV infection of precision-cut lung slices (PCLS) induced a pronounced senescent phenotype, further showcasing the translational relevance of our model. Overall, we are introducing powerful ex vivo tools which can then be utilized for the study of mesenchymal-epithelial crosstalk after IAV infection within an aged microenvironment.

H1-10: Human lung explants as a risk assessment tool for zoonotic influenza A viruses

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Avian influenza viruses (AIV) of the subtype H5Nx clade 2.3.4.4b have displayed a concerning rise in outbreak activity in wild birds and mammals. While spill-over infections of these influenza A viruses (IAV) can be accompanied by severe lung disease and a disbalanced immune response, such infections usually do not transmit between humans. However, recent outbreaks, spill-over infections and genetic reassortment events have facilitated adaptative mutations to new hosts and genetic diversification, leading to novel viruses that pose unknown pandemic and health risks to humans. To examine the potential of emerging AIV to replicate within the human lung and cause severe disease, a reliable and informative physiological model of the human respiratory tract is required.

In this context, human lung explants represent a highly valuable and structurally complex model system. Therefore, ex vivo primary human lung tissue will be employed to analyse the likelihood of infection and the kinetics of replication of emerging IAV in comparison to pre-defined growth characteristics of reference viruses. To define new risk criteria for the prediction of lung disease severity, viral immunopathology will be examined by strain-specific changes in cell tropism and pro-inflammatory cytokine transcriptome and secretome. Additionally, human lung explants will be used to assess strain-specific phosphorylation of immune-regulatory kinases to identify patterns in canonical and alternative immune signaling pathway activation that are connected to immune dysregulation and acute lung disease. Together the results should help to define lung health risks of zoonotic IAV.

To summarise, we utilize and expand primary human lung explants as an experimental basis for risk assessment of emerging, zoonotic IAV and determination of new risk criteria. Once established, this risk evaluation model could be adjusted to other pathogens, e.g. human respiratory syncytial virus, or co-infections with bacteria.

H1-11: Optimize Culture Condition for Human Alveolar Macrophages Derived from Human Monocytes and Human Induced Pluripotent Stem Cells to Study Environmental Factors of the Lung

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Alveolar macrophages (AMs) are key lung-resident immune cells that regulate tissue homeostasis and contribute to the onset and progression of various lung diseases. Their specialized functionality is shaped by environmental cues within the lung niche. Thus, generating human AMs in vitro with high fidelity to primary cells remains a major challenge. Here, we optimized culture conditions for murine AMs, human monocyte-derived AMs (MD-AMLs), and induced pluripotent stem cell-derived AMs (iPSC-AMLs), and compared their surface marker expression, metabolic features, and functional properties to primary AMs. Under optimized conditions, cells retained key features of AM identity, as demonstrated by sustained expression of CD206 and CD11c in both human and murine cells. Gene expression analysis revealed upregulation of AM-associated regulators (SPI1, PPARG, MRC1) and downregulation of matrix remodeling genes (MMP7, MMP9). Functional assays further confirmed preserved ROS production, phagocytic capacity, and cytokine release.

Together, these findings demonstrate that iPSC-AMLs and MD-AMLs recapitulate key molecular and functional characteristics of human AMs, providing a robust in vitro platform for studying disease mechanisms, environmental exposures, and developing cell-based therapies.

H1-12: Rapid matrix-free development of human iPSC-derived vascular organoids to model pediatric Pulmonary Arterial Hypertension and enable therapeutic testing

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Background – Pediatric PAH (PPAH) is poorly understood but is strongly associated with TBX4 mutations. Current therapies are mainly symptomatic, and existing models are expensive and lack clear phenotypes. We developed a rapid, matrix-free hiPSC-derived vascular organoid model with a TBX4 mutation to bridge animal models and human PPAH, investigate disease mechanisms and explore therapeutic strategies.

Method – hiPSCs were CRISPR-edited to introduce a PPAH-associated TBX4 mutation and differentiated into vascular organoids. Structure and gene expression were analyzed by 3D immunofluorescence, qPCR, bulk and scRNA-seq, and ATAC-seq, and compared to a mouse model with same mutation. Pathway-informed molecular interventions and co-culture or conditioned medium from hiPSC-derived mesenchymal stem cells (iMSCs) were tested.

Results – TBX4-mutant organoids were smaller, with reduced angiogenesis, malformed endothelial networks, and decreased PDGFRβ expression. Multi-omics analyses showed impaired developmental pathways in PDGFRβ+ cells, consistent with the mouse model findings. RNA-seq identified downregulated genes, including HAS1, which produces hyaluronic acid (HA) important for angiogenesis. HA treatment improved organoid size and endothelial networks to around 50% of WT levels. Additionally, iMSC co-culture and conditioned medium enhanced endothelial network formation and partially rescued the phenotype, supporting paracrine and niche-mediated effects.

Conclusion – These matrix-free vascular organoids contain key vascular cell types, produce endogenous matrix, and can mimic the TBX4-mutant mouse phenotype, supporting their use in disease modeling and therapeutic testing. This platform can model other TBX4 mutations and support development of pediatric-specific PH therapies.

H1-13: Between AT-2 to AT-1: Intermediate lung organoids as a model for viral infection

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Alveolar type 2 (AT-2) cells are key targets for respiratory viruses, including influenza and SARS-CoV-2. They closely interact with lung macrophages and other immune cells, thereby contributing to the coordinated antiviral and inflammatory responses during infection. In addition, AT-2 cells function as lung stem cells that can differentiate into alveolar type 1 (AT-1) cells following lung injury. Given their central role in infection and regeneration, reliable in vitro models that faithfully recapitulate the AT-2 cell phenotype are essential for studying host-pathogen interactions. Lung organoids derived from primary human AT-2 cells represent a promising model system to investigate epithelial responses to respiratory viruses and are highly susceptible to infection with both low- and highly pathogenic influenza A viruses (IAVs). However, it remains unclear to what extent these AT-2 derived organoids preserve the molecular and functional characteristics of primary AT-2 cells, or whether they adopt transitional cell states. In our group, AT-2 cells are isolated from primary human lung tissue by fluorescence-activated cell sorting (FACS) using antibodies against the epithelial cell marker CD326 and the AT-2 specific surface marker HT2-280. We performed an integrative analysis of established cell-type markers in cultured organoids by assessing their expression at both mRNA and protein levels. Compared to freshly isolated AT-2 cells, the organoids exhibit altered expression levels of key epithelial markers for adult AT-1 (PDPN) and AT-2 cells (SFTPC and LAMP3) suggesting a shift in cellular identity. Further, we observed increased expression of the AT-1 marker AQP5, alongside with downregulation but not complete loss of functional AT-2 markers (AQP1, CA2, ETV5 and WIF1). Together, these findings support that AT-2 derived lung organoids adopt a progenitor-like intermediate (AT-0) phenotype that reflects a transitional state from AT-2 towards AT-1 identity.

HUB 2

H2-1: FRED - A tool to generate FAIR metadata for omics experiments

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Scientific research relies on the transparent dissemination of data and its interpretations. This involves providing access to raw data, its metadata, details of the experimental design, and the parameters and tools used to interpret the data. The creation and management of this data presents an ongoing challenge that extends beyond publication to individual institutions, institutes, and research groups and is often referred to as research data management (RDM). It is fundamental to scientific discoveries and innovations and can be described using the terms findability, accessibility, interoperability, and reusability (FAIR). Although the majority of peer-reviewed journals require the deposit of raw data in public repositories in accordance with the FAIR principles, metadata often lacks complete standardization. This critical gap in data management practices hinders the effective use of research results and complicates the exchange of scientific knowledge. Here, we present a flexible design for a machine-readable metadata format for storing experimental metadata, along with an implementation of a generalized tool called FRED. It enables i) dialog-based creation of metadata files, ii) structured semantic validation, iii) logical search, iv) an external application programming interface (API), and v) a standalone web frontend. The tool is intended to be used by both non-computer science-trained researchers as well as specialized institutions and can be seamlessly integrated into existing RDM infrastructures.

H2-2: A pipeline for histone-wide association analysis applied to heart failure data

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Histone modifications are known for their importance for gene regulation, especially the enhancer-associated modification H3K27ac, and have been shown to be informative for predicting gene expression. Given histone data from multiple conditions, for example healthy and disease samples, a histone-wide association study (HAWAS) can be conducted, which finds genomic regions which differ between conditions. We recently proposed a model-based HAWAS, which employs pre-trained machine learning models to predict gene expression from histone data, which allows an association test directly on gene-level (10.1101/2025.05.09.653095). Here, we present an automated pipeline of this model-based HAWAS which includes the following steps: i) Generate input matrices from H3K27ac ChIP-seq bigwig-files, ii) predict gene expression with pre-trained models, iii) test for differential expression with DESeq2 including generation of diagnostic plots, iv) gene ontology and disease gene enrichment tests, v) if available, comparison to measured RNA-seq data. The pipeline is implemented with Nextflow and available on GitHub (https://github.com/schulzlab/hawas). We applied it on a patient cohort with heart failure from the MAGNet study (EGAD00001004945), to find genes that could explain the difference between failing and non-failing hearts.

H2-3: Novel statistical method for RNA-DNA interaction calling helps identify gene-regulatory RNAs in EndMT

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Chromatin-localised RNAs play diverse roles in gene regulation and nuclear architecture. Mapping genome-wide RNA–DNA interactions is possible using a variety of molecular methods, including using bridging oligonucleotides to ligate RNA and DNA in proximity. While molecular methods have progressed, a robust computational method for calling biologically meaningful RNA–DNA interactions from these data is lacking. Herein, we present RADIAnT (RNA And DNA Interactions Analysed 'n' Tested), a reads-to-interactions pipeline for the statistical analysis of RNA–DNA ligation data. RADIAnT calls interactions against a dataset-specific, unified background, which considers RNA binding site–TSS distance and genomic region bias, and outperforms previously proposed methods

in the accurate recall of genome-wide RNA–DNA interactions. Accurate RNA–DNA interaction calling enables the analysis of gene regulatory RNA sin dynamic biological contexts. Here, dynamically chromatin-associated RNAs were identified in the physiologically- and pathologically relevant process of endothelial-to-mesenchymal transition. By depleting candidate chromatin-associated lncRNAs, their gene regulatory behaviour at bound target genes important to endothelial phenotype maintenance could be validated. These data demonstrate how effective RNA–DNA interaction calling can help to place RNAs at key points in gene regulatory networks governing cellular behaviour.

H2-4: Dynamical modeling-informed interpretation of Ki67 positivity for cell cycle state determination

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The correct identification of cycling and quiescent cells is essential for cancer research. Ki67 is an intranuclear protein mostly known as a proliferation marker: both in cell imaging and flow cytometry, it is usually used as a binary proliferation versus quiescence marker, where a fixed level of fluorescence intensity separates cycling and quiescent cells. It is now established that the protein is degraded continuously in both G1 and G0 phases of the cell cycle and produced in S and G2M.

However, the consequences of this expression pattern for the interpretation of binary (Ki67 positive or negative fractions) remain largely unaccounted for, leading to systematic errors, with potentially massive over or underestimation of the quiescent fraction depending on the length of the G1 phase and the degradation kinetics of Ki67. In this work, we describe quantitatively how the percentage of Ki67 positive cells at steady state varies depending on the cell cycle dynamics.

For this purpose, we use a realistic population-level mathematical model of the cell cycle that we combine with a literature-informed model of Ki67 kinetics. Briefly, we assume that Ki67 negative cells become positive upon entering the S phase, and that Ki67 fluorescence decays continuously in G0/G1 from a uniform starting level after mitosis. Cells are expected to become Ki67 negative following a semi-deterministic delay and consequently, we model the time for cells to turn negative in G0/G1 with a gamma distribution.

With our model, we find that G0 cells mislabeled as G1 is the most prominent error under typical cell cycle parameters. We further explore various scenarios to determine how Ki67 positivity and S/G2M cells fractions are expected to change upon perturbations and identify characteristic patterns reflecting changes in single cell-cycle phases. Our work provides guidelines for both experimentalists and clinicians to interpret changes in Ki67 positivity.

H2-5: Nucleosome positioning from ATAC-seq data by NucleoDetective

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Nucleosome positioning is critical for regulation of gene expression, as nucleosomes control accessibility of regulatory DNA. While there are other assays, such as MNase-seq, that are commonly performed to examine nucleosome positioning, this information can also be inferred from ATAC-seq data. Since the Tn5 transposase is unable to cleave nucleosome-occupied DNA, structured patterns of fragments of specific sizes emerge around the protein-DNA complex. Here, we introduce NucleoDetective, a tool developed to analyze the structure of fragment patterns in sliding windows across peak regions in the genome to measure nucleosome signal. The signal is adjusted for the likelihood of nucleosome-indicating patterns emerging by chance and normalized for differences in fragment coverage between conditions. Peak regions overlapping genomic features of interest such as transcription start sites are further analyzed to describe nucleosome arrays around them to investigate chromatin structure conservation. Differential analyses are then performed to identify regions with differential nucleosome array configurations and nucleosome occupancy differences around given genomic features between conditions. Furthermore, a functional annotation of predicted nucleosomes can also be conducted. In summary, NucleoDetective is a powerful tool to paint a higher resolution picture of standard ATAC-seq analyses, allowing in-depth characterization of the accessibility and structure of gene regulatory elements and adding another dimension to epigenetic analyses.

HUB 3

H3-1: Gadobutrol Safety and Tolerability in Serial Contrast-Enhanced CMR: Prospective Data from the Myoflame-19 Randomised Controlled Trial

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Background

Contrast-enhanced CMR using gadolinium-based contrast agents (GBCAs) is integral to phenotypic characterisation of inflammatory cardiac involvement. In interventional CMR trials, participants undergo serial GBCA exposure at baseline and follow-up. Prospective tolerance data in post-COVID populations are scarce.

Purpose

To report the safety and tolerability of gadobutrol in the Myoflame-19 randomised controlled trial of cardioprotective immunomodulation for post-COVID inflammatory cardiac involvement.

Methods

Myoflame-19 enrolled 279 participants (median age 39 years, 73% women) with CMR-confirmed post-COVID inflammatory cardiac involvement and no known structural heart disease. Gadobutrol (Gadovist, 0.1 mmol/kg) was administered at baseline and week-16 CMR. The GBCA safety analysis set comprised all participants receiving at least one gadobutrol dose (n=266). AEs causally attributed to gadobutrol were coded using MedDRA and analysed separately from IMP-related AEs.

Results

A total of 508 gadobutrol administrations were performed: 266 at baseline and 242 at week 16 (ITT, n=246). Four participants did not receive gadobutrol at week 16: 2 declined, and 2 by precautionary investigator decision (not attributed to gadobutrol). Mean administered volumes were 7.40 ml (SD 1.71) at baseline and 7.24 ml (SD 2.23) at week 16. Fourteen participants received 0.2 mmol/kg in error, with no clinical sequelae. Three mild contrast-related AEs (nausea after administration) were recorded. No allergic reactions, anaphylaxis, or moderate-to-severe contrast-related AEs occurred. No contrast-induced nephropathy or nephrogenic systemic fibrosis was observed.

Conclusions

Serial gadobutrol administration was safe and well-tolerated in a post-COVID population undergoing repeat CMR within a 16-week interventional trial, supporting its use for phenotyping and serial assessment in this setting.

H3-2: Measurement strategy alters inferred age-dependent accumulation and mortality risk of mosaic Y loss

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Mosaic loss of Y chromosome (mLOY) is a widely used biomarker of biological aging, yet whether its inferred age-dependent accumulation and associated clinical risk are invariant to measurement strategy remains unclear. We compared intensity-based and phase-based quantification approaches in 223,251 men from the UK Biobank to determine how analytic definitions influence estimates of mLOY burden, risk thresholds and population prevalence. Phase-based quantification revealed a steeper and more stable age-dependent accumulation of mLOY and identified excess mortality risk at lower mosaic burdens than intensity-based metrics. These differences shifted the inferred onset of biological risk and expanded the proportion of individuals classified as affected from 5.3% to 19.2%. Conventional thresholding preferentially excluded low-burden mosaicism, compressing risk gradients and reducing statistical resolution for downstream associations. These findings show that analytic definitions materially alter inferred accumulation dynamics, risk thresholds and population prevalence of mosaic Y loss.

H3-3: Aortic Stiffness and Cardiac Inflammation, Fibrosis, and Diastolic Dysfunction in Post-COVID-19 patients

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Background

Persistent cardiovascular symptoms after COVID-19 are common, yet pathophysiological links between aortic stiffness and myocardial dysfunction remain unclear. We examined associations between central aortic PWV and markers of inflammation, fibrosis, and cardiac function in post-COVID participants without known heart disease.

Purpose

To evaluate the relationship between aortic PWV and myocardial inflammation, diastolic dysfunction, and biomarkers of systemic vascular inflammation and remodelling in a prospective post-COVID cohort at >6 months post-infection.

Methods

Prospective observational cohort of 355 symptomatic post-COVID patients (median age 43.5 years) without known heart disease undergoing comprehensive CMR including aortic PWV measurement. Biomarkers were quantified by OLINK proximity extension assay and ELISA (GPCR-autoantibodies). Pearson correlations; significance p<0.05.

Results

PWV correlated significantly with age and indices of diastolic dysfunction: E/e' mean (r=0.219, p<0.001), Native T1 (r=0.333, p<0.001), and Native T2 (r=0.16, p=0.003), indicating diffuse myocardial fibrosis and oedema. An inverse correlation was observed with global longitudinal strain (r=−0.213, p<0.001), reflecting impaired myocardial deformation. PWV correlated positively with pro-inflammatory cytokines (IL-6, MCP-1, MCP-3, MCP-4, VEGF, TNF-α) and extracellular matrix remodelling markers (Galectin-3, Galectin-9, MMP-3, MMP-10), and with the endothelial activation marker ICAM. No significant associations were found with circumferential strain, CRP, creatinine, or NT-proBNP.

Conclusions

In post-COVID participants without known heart disease, aortic stiffness associates with markers of cardiovascular inflammation and remodelling, providing mechanistic insights and potential therapeutic targets for post-COVID cardiovascular sequelae.

H3-4: Olaparib : a potential New OPTION for PAH Therapy

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Introduction: Pulmonary arterial hypertension (PAH) is characterized by progressive narrowing of distal pulmonary arteries (PAs), leading to right ventricular (RV) failure and premature death. Despite a cytotoxic inflammatory environment inducing DNA damage, PA smooth muscle cells (PASMCs) adapt and continue to proliferate. Persistent activation of the DNA damage response (DDR) drives this abnormal phenotype. Among DDR proteins, poly (ADP-ribose) polymerase (PARP) promotes aberrant DNA repair, survival, and proliferation. PARP can be inhibited by Olaparib, an orally available drug approved for ovarian cancer. PARP inhibition also improves RV function in preclinical PAH models. This study aimed to evaluate Olaparib efficacy in PAH using in vitro and ex vivo models and a Phase 1b clinical trial.

Methods and results: In PASMCs and PA endothelial cells from PAH patients, Olaparib reduced proliferation (MCM2, Ki67) and apoptosis resistance (Survivin, Annexin V). PAH-PASMCs with high baseline expression of DDR-associated proteins (RAD51, CHK2, BRCA1) showed reduced responsiveness to Olaparib compared to PAH-PASMCs with low expression of these proteins. Olaparib improved established vascular remodeling in human PCLS from PAH patients. In a Phase 1b clinical trial (OPTION, NCT03782818) involving 17 patients with severe PAH, Olaparib improved hemodynamics in 6 patients, stabilized hemodynamics in 3 patients. Post-baseline hemodynamics worsened in 3 patients and were unavailable in 5 patients due to premature discontinuation (mostly due to unrelated disease worsening).

Conclusion: Olaparib exerts anti-remodeling effects in PAH models influenced by baseline DDR activation. In patients, response was heterogeneous. Ongoing studies aim to determine whether baseline DDR activity and additional biomarkers predict hemodynamic response.