1.1 L-Citrulline transport and chronic hypoxia-induced pulmonary hypertension in newborn piglets

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Pulmonary arterial endothelial cells (PAEC) express the enzymes needed for generation of L-arginine from intracellular L-citrulline but do not express the enzymes needed for de novo L-citrulline synthesis. Hence, L-citrulline levels in PAEC are dependent on L-citrulline transport. Once generated, L-arginine can be converted to L-citrulline and nitric oxide by the enzyme nitric oxide synthase. We sought to determine whether hypoxia, a condition etiologically linked to pulmonary hypertension, alters the transport of L-citrulline and the expression of the sodium-coupled neutral amino acid transporters (SNATs) responsible for L-citrulline transport in PAEC from newborn pigs. PAEC isolated from newborn pigs were cultured under normoxic and hypoxic conditions and used to measure SNAT1,2,3, and 5 protein expression and 14C-L-citrulline uptake. SNAT1 protein expression was increased, while SNAT2, SNAT3, and SNAT5 expressions were unaltered in hypoxic PAEC. 14C-L-citrulline uptake was increased in hypoxic PAEC. Studies with inhibitors of System A (SNAT1/2) and System N (SNAT3/5) and with knockdown of SNAT1 by silencing RNA technique revealed that the increased 14C-L-citrulline uptake in hypoxic PAEC was due to the System A transporter, SNAT1. In additional studies we evaluated SNAT protein expression and L-citrulline levels in lungs of piglets with chronic hypoxia-induced pulmonary hypertension and comparable age controls. Lungs from piglets raised in chronic hypoxia exhibited greater SNAT1 expression and higher L-citrulline levels than lungs from controls. Our findings indicate that increased SNAT1 expression and the concomitant enhanced ability to transport L-citrulline in PAEC could be important mechanisms to counteract NO signaling impairments known to occur during the development of chronic hypoxia-induced pulmonary hypertension in newborns. Therapeutic manipulation of SNAT1 or L-citrulline transport may have therapeutic benefit in neonatal patients with pulmonary hypertension.

1.2 Umbilical cord blood angiogenic progenitor cells are decreased in moderate and severe bronchopulmonary dysplasia

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Bronchopulmonary dysplasia (BPD), the chronic lung disease of prematurity, is associated with impaired vascular and alveolar growth. Disrupted angiogenesis results in a simplified lung structure that predisposes preterm infants to postnatal pulmonary hypertension. Antenatal factors contribute to the risk for developing BPD by unclear mechanisms. Endothelial progenitor cells (EPCs), such as angiogenic circulating progenitor cells (CPCs) and late-outgrowth endothelial colony-forming cells (ECCFs), may contribute to angiogenesis in the developing lung. Yet whether disruption of EPC function contributes to the pathobiology of BPD is unknown. We hypothesize that cord blood angiogenic CPCs and ECCFs are decreased in preterm infants with moderate and severe BPD. We quantified ECCFs in culture and utilized polychromatic flow cytometry to measure the CPC-to-non-angiogenic-CPC ratio (CPC: non-CPC) in cord blood samples from 62 preterm infants. We then assessed their relationships to maternal and perinatal risk factors as well as BPD severity. The CPC: non-CPC ratio and ECCF number were compared between preterm infants with mild or no BPD and those with moderate or severe BPD. ECCF number (P < 0.001) and CPC: non-CPC ratio (P < 0.05) were significantly decreased in cord blood samples of preterm infants who subsequently developed moderate or severe BPD. ECCF number but not the CPC: non-CPC ratio was significantly increased in infants with chorioamnionitis (P < 0.01) and vaginal birth (P < 0.01). Gestational age and birth weight were not associated with either angiogenic marker. Circulating vascular progenitor cells are decreased in the cord blood of preterm infants who develop moderate and severe BPD. These findings suggest that prenatal factors contribute to late respiratory outcomes in preterm infants. We speculate that EPC disruption results in a simplified pulmonary vascular bed that leads to late pulmonary hypertension in preterm infants with severe BPD.

1.3 Subcellular mechanisms in IPAH-dysfunctions of the Golgi apparatus/endoplasmic reticulum/mitochondrial axis

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In 1977, Smith and Heath reported using EM methods the marked cystic dilatation of the endoplasmic reticulum (ER) in PAECs in hypoxic rats with PAH, and in 1979 they reported dilatation of the ER cisternae in cells in vascular lesions of PAH in man. In recent years, we reported Golgi apparatus enlargement and fragmentation, increased cytoplasmic dispersal of the Golgi tether giantin and defects in intracellular trafficking leading to global changes in the cell surface landscape of pulmonary vascular cells within PAH lesions in man and PAH-like lesions in SHIV, but not SIV, infected macaques. In observations that now unite data concerning Golgi, ER and mitochondrial changes in PAH, we demonstrate that siRNA-mediated acute knockdown of STAT5a/b species in pulmonary vascular cells led to Golgi enlargement and fragmentation, cystic dilatation of ER, increased VTCN1 expression, and reduced TMRE uptake. IPAH-derived mutant BMPRII species inhibited ER to Golgi plasma membrane trafficking combinatorially with downstream regulation of STAT5a or STAT5b or eNOS. Increased RTN4 in cystic cells with distorted nuclei and mislocalized STAT5a was also observed in arterial walls, and increased RTN4 and ATL3 in cells lining the channels.
in plexiform/obliterative lesions in IPAH and in SHIV-infected macaques and in thickened alveolar septa. Single-cell 3D imaging revealed that eNOS was largely trapped in intracellular structures, including the Golgi, in cells in IPAH lesions. Taken together with the fact that that STAT5α/b species are known to be estrogen responsive, the data point to novel intracellular organelles mechanisms that might underlie second-hit, low-penetration and sexual dimorphism issues in PAH. Importantly, the new data highlight the EM observations of Smith and Heath of 1977 and 1979. Supported by Research Grant R01 HL07176 from NIH, NHLBI.

1.4 Endostatin (Col18a1) as a novel determinant of disease susceptibility and severity in pulmonary hypertension

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Pulmonary arterial hypertension (PAH) is a progressive syndrome leading to right ventricular failure (RVF) and death. Altered vascular tone/remodeling increases right ventricular (RV) afterload resulting in adaptive hypertrophy (RHH) and/or RVF. During adaptive hypertrophy, ventricular angiogenesis increases to match demand and preserve perfusion. Loss of capillary density (rarefaction) has been linked with ventricular dysfunction. Both the molecular regulators of RV angiogenesis/vasculogenesis and rarefaction and their contribution to RV performance are incompletely defined. Endostatin is a potent angiostatic protein derived from the proteolytic cleavage of collagen 18A1 (encoded by the Col18a1 gene). Using both DNA microarray and quantitative PCR we demonstrate that Col18a1 mRNA increases in the RV of mice challenged with chronic hypoxia (CH) and rats exposed to Sugen coupled with CH (Sugen/CH), models of RVH and RVF, respectively. Further, treatment with the PDE5 inhibitor Sildenafil prevents this increased expression in response to Sugen/CH coincident with improved RV performance. Analysis of circulating endostatin demonstrates increased serum endostatin in a murine model of PH (CH-PH) and in human idiopathic (IPAH) and scleroderma associated PAH (SSc-PAH). Moreover, genetic analysis of single nucleotide polymorphisms (SNPs) in Col18a1 link distinct polymorphisms with altered risk of developing PAH and/or with worse hemodynamics, specifically decreased cardiac index and/or increased pulmonary vascular resistance. Thus, using both preclinical animal models of RVH and RVF in PH and clinical serum, genetic, and hemodynamic analysis in human IPAH and SSc-PAH, we have identified endostatin/Col18a1 as a novel determinant of disease susceptibility and severity in PAH and a potential target for therapeutic intervention.

1.5 Computed tomographic assessment of pulmonary vascular remodeling in smokers

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As ascertained by early roentgenologic investigation, angiography, and necropsy, there are several types of pathologic pulmonary vascular remodeling possible in smokers. These range from inflammatory remodeling with progressive luminal occlusion, aberrant vessel elongation in regions of hyperinflation, and outright loss of vasculature in regions of severe emphysematous destruction. We hypothesized that such pathologic morphologies could be objectively assessed on CT scan and are associated with clinical manifestations of smoking related lung disease. Using imaging and clinical data from the COPDGene Study we created lobe specific volumetric models of the intraparenchymal pulmonary vasculature and objectively assessed vessel dilation, pruning, elongation, and dropout. Vessel dilation (blood volume of vessels 10-15 mm) was related to emphysema defined by a Hounsfield Unit threshold of -950 (n = 2254; R = 0.17, P < 0.0001 for right upper lobe - RUL, R = 0.13, P < 0.0001 right lower lobe - RLL) and to PA pressure estimated by echo (n = 77; RUL: r = 0.22, P = 0.03, RLL: r = 0.22, P = 0.03). In 147 subjects, distal pruning (blood volume in vessels <5mm2) was related to DLCO (RUL: R = 0.3, P < 0.0001, RLL: R = 0.43, P < 0.0001). Hyperinflation (CT TLC%) and emphysema were directly related to vessel length (n = 192; r = 0.36, P < 0.0001 and r = 0.30, P < 0.0001 respectively) and inversely related to vessel number (n = 192; r = -0.22, P = 0.05; r = -0.53, P < 0.0001). Vessel number was directly related to 6 min walk distance (6 MWD: r = 0.45, P < 0.0001). Quantitative CT based assessments of intraparenchymal vessel dilation, pruning, elongation, and drop out provides clinically relevant information in smokers.

1.6 Potential biomarkers in pulmonary arterial hypertension associated with limited scleroderma

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Pulmonary arterial hypertension (PAH) is a common complication in limited systemic sclerosis (1SSc). Identification and characterization of biomarkers for 1SSc-PAH could lead to less invasive screening, better understanding of pathogenesis, and improved treatment. Forty-nine PBMC samples were obtained from 21 1SSc subjects without PAH (1SSc-noPAH), and 10 healthy controls. Genome-wide gene expression was measured for each sample. Levels of 89 cytokines were measured in serum from a subset of subjects by Multi-Analyte Profiling (MAP) immunoassays. Gene expression clearly distinguished 1SSc samples from healthy controls, and separated 1SSc-PAH from 1SSc-NoPAH patients. RT-PCR confirmed increased expression of nine genes (ICAM1, IFNγR1, IL13Ra1, JAK2, AIF1, CCR1, ALAS2, TIMP2) in 1SSc-PAH patients. Increased circulating levels of inflammatory mediators, such as TNF-alpha, IL 1-β, ICAM-1, and IL-6, and markers of vascular injury, such as VCAM-1, VEGF, and vWF were found in 1SSc-PAH subjects. In further studies, increased PBMC expression of IFN-γ regulated and biomarker cluster, but not in the IFN-regulated cluster, distinguished 1cSSc-PAH from 1cSSc-noPAH. The genes CCR1 and JAK2 were expressed more highly in 1cSSc-PAH than in controls and mainly by CD14+ cells. MRC1 expression was increased exclusively in 1cSSc-PAH and correlated strongly with PAP and increased mortality. IL-13 concentration was most increased in 1cSSc-PAH. Gene expression and cytokine profiles of 1SSc-PAH patients suggest presence of activated monocytes and show markers of vascular injury and inflammation. IFN-regulated and biomarker genes represent distinct, although related, clusters in 1cSSc-PAH. MRC1 is associated with PAH and related to mortality. These genes and factors could serve as biomarkers of PAH in 1SSc.

1.7 Pulmonary arterial hypertension in a non-human primate model of HIV

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Non-human primates infected with simian immunodeficiency virus (SIV) or humanized SIV (SHIV) exhibit histologic changes characteristic of human PAH, suggesting that the pathologic changes seen in this pathologic is unknown. As histologic changes occur in only a percentage of SIV or SHIV-infected animals and development cannot be predicted prior to necropy, measuring pulmonary
artery pressures would facilitate following disease over time. To determine the effect of SHIV and SIV infection on right heart and pulmonary arterial pressures in a macaque model of HIV-associated PAH. We performed right heart catheterizations (RHC) in 19 immunosuppressed macaques (Macaca fascicularis) infected with SHIV89.6P, and compared right heart and pulmonary artery pressures to those in 4 uninfected control animals. We also performed RHC in 4 Rhesus macaques (Macaca mulatta), pre- and at 8 months post-infection with SIVdeltaB670. Right atrial, right ventricular systolic, and pulmonary artery pressures were significantly elevated in SHIV-infected animals compared to controls with no difference in pulmonary capillary wedge pressures. Pulmonary vascular resistance was elevated in SHIV infected animals. Cardiac output was similar to controls. SHIV-infected macaques had modest histologic changes in pulmonary arteries. Serial RHC data in Rhesus macaques revealed significant elevation in RSP at 8 months post-SIV infection. This is the first report of pulmonary hemodynamic consequences of SHIV and SIV infection in macaques and establishes a powerful tool for following disease development and efficacy of interventions in a longitudinal model.

1.8 Efficacy and safety of imatinib in the treatment of pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH), characterized by pulmonary vasculature proliferation and increased pulmonary vascular resistance (PVR), results in death from right ventricular failure. We assessed the efficacy and safety of imatinib in patients with PAH and PVR ≥800 dyne·s·cm⁻⁵ despite ≥2 PAH medications. Patients (n = 202) were randomized to imatinib or placebo for 24 weeks (core study), with the option to participate in an open-label extension (ongoing). In the core study, the primary outcome was change in 6-min walking distance (6MDW); secondary outcomes included change in hemodynamics and time to clinical worsening (TTCW). Imatinib improved 6MDW (+32 m, P = 0.002), PVR (-379 dyne·s·cm⁻⁵, P < 0.001) and cardiac output (+0.88 L/min, P < 0.001) at Week 24 compared with placebo. There was no significant difference in TTCW (P = 0.563). In patients who remained on imatinib in the extension (n = 66), improvements in 6MDW were maintained at week 24 of the extension (48 weeks imatinib [n = 54]). Five patients in each group died during the core study; 6 patients died in the extension, all received placebo in the core study. AE’s were typical for imatinib in the core study, particularly in the first eight weeks of treatment, and included nausea, vomiting, diarrhea and peripheral edema. More imatinib patients discontinued the core study (33% vs. 18%). Unexpectedly, subdural hematoma (SDH) occurred in eight patients (two of 103 imatinib patients in the core study [1.9%]; 6 of 144 patients in the extension [4.2%]), all in patients on imatinib and anticoagulation. Five patients with SDHs recovered, one died of SDH and two died of unrelated causes. The extension study is ongoing; efficacy and safety assessments continue every six months to provide additional data regarding benefits and risks of imatinib in advanced PAH. Imatinib is currently not approved for treatment of PAH.

1.9 MTORC2 regulates vascular smooth muscle cell metabolism in pulmonary arterial hypertension

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Abnormal proliferation of pulmonary arterial vascular smooth muscle cells (PASMC) coupled with a metabolic shift to glycolysis are key pathological components of vascular remodeling in PAH. mTOR is a central controller of cell growth, proliferation and survival. mTOR acts through rapamycin-sensitive mTORC1 (mTOR-raptor, modulating cell growth) and rapamycin-resistant mTORC2 (mTOR-rictor, activating Akt). The role of mTORC2 in PASMC proliferation, however, is not clear. We found that mTORC2-Akt is up-regulated in distal PASMC from patients with idiopathic PAH (iPAH) and from rats with chronic hypoxia-induced PH as shown by immunohistochemical and immunoblot analysis with P-S2481 mTOR and P-S473 Akt antibodies. Human iPAH and rat PH PASMC had increased cellular ATP levels (254 ± 18.2% and 1294 ± 5.8% vs. 100% for control cells; P < 0.01) and proliferation (DNA synthesis) versus controls (P < 0.001). There was a significant increase of anti-apoptotic Bcl2 and deficiency of proapoptotic Bim in human iPAH PASMC. 2-DG, but not rotenone, significantly reduced ATP content, proliferation, and induced apoptosis in human iPAH and rat PH, but not in control PASMC. sRNA rictor and Akt1/2 inhibitor markedly decreased ATP levels and reduced human iPAH and rat PH PASMC proliferation via activating energy sensor AMPK. sRNA rictor significantly decreased Bcl2 and elevated Bim protein levels and apoptosis in iPAH PASMC but not in controls (P < 0.01). These data show that mTORC2-Akt is required for glycolytic energy production, proliferation and survival of human iPAH and rat PH PASMC and suggest that mTORC2-Akt represents a potential therapeutic target for patients with PAH. Funded by: Gilead Science Research Scholars Grant (E.A.G.), American Lung Association Research Grant (E.A.G.), University Research Foundation Grant (E.A.G.).

1.10 Schistosomiasis-induced pulmonary vascular disease is IL4/IL13 and TGF-β dependent

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Schistosomiasis infects over 200 million people worldwide, and an estimated five to 15 million individuals have schistosomiasis-associated pulmonary arterial hypertension (PAH). The pathogenic mechanism linking infection and the immune response to vascular remodeling is unknown, but may be similar to other forms of inflammatory PAH such as idiopathic and connective-tissue disease associated. We sought to develop and characterize an animal model of schistosomiasis-induced pulmonary hypertension, and compare the features to human lung tissue collected at autopsy from individuals with the disease in Brazil. Mice experimentally infected with Schistosoma mansoni can have an increase in right ventricular systolic pressure, pulmonary vascular remodeling, and right ventricular hypertrophy. There is activation of the IL4, IL6, IL13, and TGF-β signaling pathways. Mice lacking the canonical IL13 receptor IL13R1c1 are only mildly protected while mice lacking both IL4 and IL13 are more completely protected, suggesting IL4 and IL13 can be redundant for each other. S. mansoni-infected mice have an IL4/IL13-dependent increase in TGF-β signaling. Inhibition of TGF-β with a neutralizing antibody blocks the vascular remodeling, but also decreases IL4 and IL13 activity. IL6 activation is decreased by either the absence of IL4 and IL13 or the inhibition of TGF-β. The IL3 target RELM-α, associated with other models of pulmonary hypertension, does not stringently correlate with vascular remodeling. In the human lung autopsies, TGF-β and IL6 but not RELM-α signaling is present. Mice experimentally infected with S. mansoni can have multiple features consistent with pulmonary hypertension and reproduce key aspects of the human disease. The inflammation-induced pulmonary vascular remodeling appears to be dependent on a feedback loop of IL4/IL13 and TGF-β signaling, and suggests potential opportunities for therapeutic intervention.
1.11 BMPR2 alternative splicing and PAH: A molecular explanation for gender discrepancy?

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Germline BMPR2 mutations constitute the largest known risk for developing PAH. One of the most definitive, but poorly understood, modifiers of PAH is gender. Female BMPR2 mutation carriers are more likely to develop PAH than men though the molecular basis of this gender preference remains unknown. Here we provide evidence that this may in part be due to BMPR2 alternative splicing. The BMPR2 gene has 13 exons and is alternatively spliced to produce two primary transcripts: (1) isoform-A, which is the full length BMPR2 gene product and contains all 13 exons; and (2) isoform-B, which is a less abundant and is missing exon-12. Studies have shown that exon-12 is important for proper functioning of BMPR-II receptor; BMPR2 gene product that is missing exon 12 can act in a dominant negative fashion to inhibit BMPR-II receptor function. Here we tested the hypothesis that females have higher expression of isoform-B compared to isoform-A (higher B/A ratio). Using real-time PCR we determined isoform levels in cultured lymphocytes from 50 females and 33 males (all BMPR2 mutation carriers). We found that indeed females had higher B/A ratios than males (P < 0.01). Given multiple observations suggesting that estrogens may modify development and course of PAH, we further hypothesized that estrogens directly regulate BMPR2 B/A isoform ratio. We exposed PMVECs to various doses of estradiol and found estrogen exposure increased B/A isoform ratio. Conclusions: (1) Females have higher B/A isoform ratios than males. (2) Exposure to estrogens increases the B/A ratio in PMVEC cells. Differential BMPR2 isoform ratios, modified by estradiol, may provide a molecular mechanism that could partially explain the higher incidence of PAH among females.

1.12 Thrombospondin-1 inhibits proliferation of pulmonary vascular smooth muscle and endothelial cells: A mechanism for loss-of-function thrombospondin-1 mutations as modifiers in familial PH

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Carriers of BMPR2 mutations in familial pulmonary arterial hypertension (PAH) cohorts have only a 20% risk of disease, suggesting that other factors influence the development of PAH in these individuals. Thrombospondin-1 (TSP1) regulates activation of TGF-β and inhibits endothelial and smooth muscle cell proliferation - processes key to PAH pathophysiology. To assess a role for TSP1 in familial PAH risk (and PAH pathogenesis) we resequenced the regions of the TSP1 gene (THBS1) encoding the TGF-β and cell growth inhibition domains in 60 FPAH probands and three control groups. We identified THBS1 mutations in three families: A novel missense mutation in two (Asp362Asn), and an intronic mutation in a third (IVS8 + 255 G/A). Neither mutation was observed in control cohorts. Recombinant mutant 362Asn TSP1 had <50% of wild-type TSP1’s ability to activate TGF-β (P < 0.01). Mutant 362Asn TSP1 also lost the ability to inhibit pulmonary arterial smooth muscle cell (PASMC) growth, and was over 3-fold less effective at inhibiting endothelial cell growth (P < 0.001). The vascular receptors thought primarily responsible for TSP1 effects are CD36 and CD47. Interestingly, the effects of exogenous TSP1 appeared biphasic - low pM concentrations of TSP1 augmented PASMC growth in vitro, and this could be blocked by CD47 (but not CD36) inhibition by specific antibodies (P < 0.05). The higher (nM) concentrations of TSP1 that led to growth inhibition in PASMC did not involve CD47 (based on antibody blocking studies), suggesting that CD36 or other receptors are responsible for these inhibitory effects. While THBS1 mutations in familial PAH have implications in the genetic evaluation of PAH patients, they also highlight a likely role for TSP1 in the pathogenesis of PAH through its effects on TGF-β and vascular cell growth. The specific receptors and signaling pathways by which TSP1 inhibits pulmonary vascular cell growth remain to be determined.

1.13 In vivo circulating fibrocytes ablation in the setting of pulmonary hypertension murine model

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Circulating fibrocytes are circulating mesenchymal cells originating from the peripheral blood/bone marrow. Several recent reports showed the recruitment of circulating fibrocytes in fibrotic or remodelled tissue in various pathological conditions. At time of injury, a signal triggers the release of precursor cells of fibrocytes from bone marrow to the circulation, followed by their recruitment to the injured tissue. As circulating fibrocytes have been implicated in the pathogenesis of pulmonary diseases but their direct contribution in remodelling process is not clear. So we want to specifically target the circulating fibrocytes to ablate them using suicidal gene strategy. Transgenic mice were generated in which herpes simplex virus thymidine kinase (HSV-TK) was expressed under the control of collagen1α2 promoter. The key feature of circulating fibrocytes is the collagen1 expression and therefore circulating fibrocytes will express HSV-TK. The chronic administration of ganciclovir (GCV, antiviral medicine) to the transgenic mice carrying HSV-TK gene under collagen1α2 promoter will selectively ablate cells with collagen1 expression. To target exclusively blood/bone marrow derived, circulating fibrocytes and not fibroblast, we performed bone marrow transplantation i.e., we transplanted bone marrow from transgenic HSV-TK positive mice to wild type mice. After transplantation these mice were kept for constitution of their bone marrow for four to six weeks and these mice were subjected for hypoxia for five weeks to develop pulmonary hypertension. To ablate the circulating fibrocytes in vivo, we administered the GCV as preventive measure (either at the time of bone marrow transplantation or at the time of disease induction) and as therapeutic measure i.e., after development of disease. We observed that there was slight reverse remodelling in the right ventricular hypertrophy without changing the right ventricular systolic pressure. The vascular remodelling was significantly reduced with circulating fibrocytes ablation as seen with reduced vessel wall thickness. The number of recruited collagen1αCD45+ were significantly reduced as assessed by flow cytometry and immunostaining. Taken together, our data suggests that circulating fibrocytes have severe impact in the progression of disease and their in vivo ablation positively regulated the vascular remodelling.

1.14 Therapeutic targeting of microRNAs in pulmonary hypertension

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MicroRNAs (miRs) control various cellular processes in tissue homeostasis and disease by regulating gene expression on the posttranscriptional level.
Recently, it was demonstrated that the expression of miR-21 and members of the miR-17-92 cluster was significantly altered in experimental pulmonary hypertension (PH). We intend to evaluate the therapeutic efficacy and anti-remodeling potential of miR inhibitors in the pathogenesis of PH. We first tested the effects of miR inhibitors (antagomirs), which were specifically designed to block miR-17 (A-17), miR-21 (A-21) and miR-92a (A-92a) in chronic hypoxia-induced PH in mice. A-17 and A-21 reduced right ventricular systolic pressure (RVSP) and all antagomirs decreased pulmonary arterial muscularisation. However, only A-17 reduced hypoxia-induced right ventricular hypertrophy and improved pulmonary artery acceleration time (PAAT). Therefore, we additionally tested the effects of A-17 in monocrotaline-induced PH in rats. A-17 treatment significantly decreased RVSP and total pulmonary vascular resistance index, increased PAAcT, normalized cardiac output and decreased pulmonary vascular remodeling. Among the tested miR-17 targets, the cyclin-dependent kinase inhibitor 1A (p21) was upregulated in lungs undergoing A-17 treatment. Likewise, in human pulmonary artery smooth muscle cells, A-17 increased p21. Overexpression of miR-17 significantly reduced p21 expression and increased proliferation of smooth muscle cells. Our data demonstrate that A-17 improves heart and lung function in experimental PH by interfering with lung vascular and right ventricular remodeling. The beneficial effects may be related to the upregulation of p21. Thus, inhibition of miR-17 may represent a novel therapeutic concept to ameliorate disease state in PH.

1.15 FK506-identified in a high throughput screen to increase BMP2 signaling-reverses pulmonary hypertension by rescuing endothelial dysfunction

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Reduced expression of BMP2, even without a loss of function mutation, seems to be a feature in many forms of pulmonary arterial hypertension (PAH). We hypothesized that increasing BMP2 signaling in PAH might rescue endothelial dysfunction and reverse disease. 3600 FDA approved drugs were screened for their propensity to activate BMP signaling, using C2C12 cells expressing a BMP response element (BRE) from the Id1 promoter fused to the luciferase-gene (BRE-luc). We tested in cell culture whether the best "hit" would rescue BMP2 signaling as well as endothelial cell function in control and PAH Pulmonary Artery (PA) ECs. We then assessed whether the BMP2 activator could also reverse established PAH in an experimental rat model of severe pulmonary hypertension that mimics clinical disease (Sus416/hypoxia/normoxia). FK-506 (tacrolimus) was found to be the main kinase signaling pathway. Unfortunately, although a relation between PAH and metabolic syndrome has been proposed, the interaction of these two conditions has not been convincingly demonstrated. We use hypertensive, diabetic, insulin-resistant, obese rats (ZSF1) and create PAH through a subcutaneous injection of vascular endothelial growth factor receptor blocker SU-5416 (sugen, 100 mg/kg). We detect increases in mean arterial blood pressure, right ventricular systolic pressure (RVSP) and lungs weight in ZSF1 treated with sugen (P < 0.05). More importantly, a significant interaction between treatment and obesity was detected (P < 0.05) in regard to RVSP and lungs weight. After developing a new model for an interaction between PAH and metabolic syndrome, we evaluated the effects of dietary supplementation with nitrite, which has been proposed as therapy for PAH by acting as a reservoir for NO, or with the AMP-kinase activator, metformin. In order to evaluate the effect of nitrite therapy in obesity and pulmonary hypertension, ZSF1 (Ob) rats and their lean (Ln) littersmates were injected with vehicle (Ob-CTL or Ln-CTL) or sugen (Ob-PH or Ln-PH). Within obese groups, sodium nitrite was administered through the water at two different concentrations (50 mg/L and 100 mg/L) and metformin at (300 mg/kg animal). Fourteen weeks into nitrite treatment, there were significant decreases in fasted and fed glucose levels, glycated hemoglobin (Hba1c) levels and oral glucose tolerance (OGT) between nitrite treated and non-treated groups in the Ob-CTL (P < 0.05), whereas no effect of nitrite on the metabolic syndrome was observed in the Ob-PH rats. Compared to non-treated obese animals, treatment with sodium nitrite decreased right ventricular peak systolic pressure (P < 0.005). Metformin improved metabolic syndrome and reduced pulmonary pressures in both the Ob-CTL and the Ob-PH groups. These studies provide clear evidence for a significant interaction between metabolic syndrome and acute VEGF inhibition with sugen to produce pulmonary hypertension, providing a new model of metabolic syndrome and PH. Chronic oral therapy with nitrite improves metabolic syndrome in obese rats and reduce pulmonary pressures in the animals with metabolic syndrome and PH. This study confirms recent findings suggesting that nitrite can have a therapeutic effect in treating PH and metabolic syndrome.
1.18 Changes in right ventricular function in a mouse model of severe pulmonary hypertension

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Pulmonary arterial hypertension (PAH) is the most severe form of pulmonary hypertension due to its rapid progression to right ventricular (RV) failure. Until the recent combination of chronic hypoxia with antiproliferation treatment by SUS416, there was no mouse model for severe or irreversible PAH. This new model (HySu) recapitulates hallmarks of human PAH, especially distal arteriolar neoinnervation and obliteration. However, the changes in RV function in this model have not been examined. Here we investigate the hypothesis that the HySu mouse model mimics the progression of RV dysfunction from compensatory to maladaptive remodeling found in PAH clinically. Male C57BL6/J mice were exposed to normoxia or 14 days or 21 days of normobaric hypoxia (10% O2). Mice exposed to hypoxia were injected once weekly with SUS416 (20 mg/kg, i.p.); normoxic mice were injected with vehicle (CMC solution). After anesthesia, in vivo hemodynamics and RV function were measured by catheterization with a 1.2F admittance pressure volume catheter with normoxic (21% O2) and hypoxic (8% O2) ventilation. Pressure-volume loops were recorded using commercially available software. All procedures were approved by the UW IACUC. Statistics were performed using 1-way ANOVA for exposure at each ventilation condition. Consistent with a previous report (Ciucan et al., 2011), RVSP increased with HySu to generate moderate (14-day) and severe (21-day) PAH. Fulton index and hematocrit increased with moderate PAH but did not increase further with severe PAH. RV contractility (dP/dtmax) increased with PAH but compliance decreased. Ees tended to increase with moderate PAH but then plateaued as the PAH became severe. Ea and total PVR increased with severe PAH. As a result, the ventricular vascular coupling efficiency (Ees/Ea) tended to increase with moderate PAH and return to control levels in severe PAH, suggesting that RV remodeling may shift from adaptive to maladaptive with severe PAH, which would mimic changes in RV function with PAH progression found clinically.

1.19 Loss of tolerance associated with bronchus associated lymphoid tissue expansion in experimental pulmonary hypertension

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Pulmonary hypertension (PH) is characterized by inflammatory cell recruitment, altered inflammatory cytokine profiles, and often, the presence of a bronchus-associated lymphoid tissue (BALT). Tertiary lymphoid tissues are implicated in a variety of autoimmune diseases, and bronchus associated lymphoid tissues (BALT) are present in the setting of PH in both humans and in several animal models. We hypothesized that in PH, lung injury with subsequent presentation of a high load of self-antigens may present an overloading burden to the “maintenance of self” apparatus of the BALT leading to the development of autoimmune-related vascular remodeling. To test our hypothesis, we used immunohistology, laser-capture microdissection, qPCR, cell culture, immunoblot, and enzyme-linked immunosorbent assay. We found increased numbers of BALT that were larger and more proliferative (Ki67+) in rats with PH, in both hypoxia and monocrotaline (MCT) models, compared to controls. In PH rats but not controls, we observed distinct segregations of T and B cells within BALT, antibody class-switching (AID), and complex lymphatic and vascular networks. These networks were characterized by high expression of peripheral node addressin, CCL19, and CCL21. In and around airways and BALT, we found distinct populations of CCR7+, CD11c, OX-62, and CD11b+ dendritic cells that co-expressed CD86, a marker of dendritic cell activation. Results were confirmed by microdissection and qPCR of BALT. Plasma from MCT rats, but not controls, contained autoabs that bound adventitia and media of rat lung sections. Interestingly, the presence of autoabs was evident at one week post-MCT injection, preceding vascular remodeling and increased PH. Plasma from four week MCT rats immunoblotted not only lung lysates, but also heart and skeletal muscle, indicating inter-epitope humoral autoimmune spreading. Tissue staining results were confirmed by rat lung tissue immunolabeling of fluo-conjugated vimentin, HSP27, and PI3kinase, identified previously by other groups as autoantigens in human PH. In addition, rat plasma autoabs readily labeled cultured control rat pulmonary artery fibroblasts and, to a lesser extent, pulmonary artery smooth muscle cells. Autoab binding induced IL-6 and MCP-1 production and secretion by fibroblasts. In vivo pharmacologic and immune-based blockade of BALT cell effectors (either CCR7 or LTBR) worsened PH and remodeling, while blockade of lymphangiogenesis and macrophages (VEGF-R3) or modulation of the unfolded protein response (salubrinal) attenuated both the PH and the presence of plasma autoabs. BALT activation and expansion are associated with loss of tolerance in experimental PH in a manner consistent with the pathobiology of human PH. Understanding the mechanisms of self-tolerance and its potential loss in PH may therefore be of novel clinical significance.

1.20 Impaired pulmonary angiogenesis in idiopathic pulmonary arterial hypertension is linked to abnormal pericyte function and reduced endothelial-pericyte interactions

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Idiopathic pulmonary arterial hypertension (IPAH) is associated with progressive small vessel loss and impaired vascular regeneration. Pericytes are highly specialized vascular cells that directly interact with endothelial cells to provide mural support and promote small vessel maturation and survival. While it is possible that abnormalities in the initiation and maintenance of endothelial-pericyte interactions could contribute to small vessel loss in IPAH, little is known about the role of pericytes in the pulmonary circulation given the lack of available methods to purify these cells from the lung. To circumvent this limitation, we have developed a novel method using 3G5 IgM coated magnetic beads to purify pericytes from the lungs of failed donors and IPAH patients obtained through the pulmonary hypertension breakthrough initiative (PHBI). All cultures obtained were >95% pure as confirmed by immunofluorescence (IF) using pericyte specific markers. Co-culture studies of healthy pulmonary artery endothelial cells (PAECs) on matrigel demonstrated reduced vascular tube formation in the presence of IPAH pericytes compared to control (P < 0.0001). Given that the vast majority of IPAH pericytes failed to reach and attach themselves to endothelial tubes, we sought to determine whether abnormalities in adhesion, motility and cell polarity were present. In support of an adhesion defect, we found that IPAH pericytes demonstrated a more disorganized actin cytoskeleton and fewer focal adhesions compared to healthy controls. Furthermore, motility studies using a modified Boyden chamber co-culture assay also demonstrated that transmigration of IPAH pericytes either alone or in response to incubation with PAECs was decreased (P < 0.001). Interestingly, we also found that reduced motility was directly correlated to failure to establish proper polarity towards the PAEC monolayer as evident in a modified scratch assay (P < 0.001).

Previous studies by our group have demonstrated that disruption of WNT/planar cell polarity (PCP) can contribute to both PAEC loss and impaired tube formation supporting a critical role for this pathway in pulmonary angiogenesis. Given the discrete adhesion, motility and polarity defects of IPAH pericytes, we are now proposing that disruption in WNT/PCP pathway could contribute to the phenotypic differences observed between healthy and IPAH pericytes. To begin exploring this possibility, we have begun characterizing gene expression pattern of WNT ligands and receptors in
healthy pericytes and PAECs alone and after co-culture using SVR green quantitative-PCR. Compared to single cultures, WNT 5a gene expression was increased in PAECs while an increase in both Vangl 1 and ROR2 were seen in pericytes. Given that WNT 5a is a known ligand of WNT/PCP that triggers intracellular signaling through the surface receptors Vangl 1 and ROR2 receptors, these preliminary studies strongly suggest that WNT/PCP pathway may be involved in the establishment of PAEC-pericyte interactions during normal pulmonary angiogenesis.

Future studies will explore how abnormalities in the activation of the WNT/PCP pathways could impair the effort of pericytes and PAECs to regenerate pulmonary vessels in response to injury and compare patterns of WNT/PCP activation between healthy and IPAH pericytes in an effort to better understand the mechanisms of small vessel loss in IPAH.

### 1.21

**Macrophage migration inhibitory factor: A mediator of hypoxia-induced pulmonary hypertension**

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PH is a devastating disease leading to progressive hypoxemia, right ventricular failure, and death. Hypoxia can play a pivotal role in PH etiology, inducing pulmonary vessel constriction and remodeling. Our studies have focused on the inflammatory cytokine MIF and its role in the development of hypoxia-induced PH. Previously, we identified the lung as a major source of MIF, and thymoxine (T₃) as a natural inhibitor of its inflammatory activities. We have now shown that plasma MIF, in patients with primary PH, or PH secondary to interstitial lung disease (ILD), is significantly higher than in controls, and is increased following exercise overy. In vitro, we found a) hypoxia increases MIF mRNA, extracellular MIF protein accumulation and vascular cell proliferation; and b) ISO-92, a small molecule inhibitor of MIF inflammatory activity, reduced hypoxia-induced cell proliferation. Furthermore, MIF-T₃ interactions altered activation of MAPK pathways in primary human pulmonary endothelial cells. In our animal model using C57BL/6 mice exposed to normobaric 10% oxygen, ISO-92 administered during hypoxic exposure, significantly reduced pulmonary vascular remodeling, right ventricular hypertrophy and pulmonary hypertension. In addition, ISO-92 attenuated hypoxia pulmonary vascular muscularization in the small sized vessels (diameter <30 µm). The data suggest that MIF plays a critical role in hypoxia-induced PH, and its inhibition beneficial in preventing development the disease. Since T₃ is a natural inhibitor of MIF, further studies should explore the effects of interactions between the two molecules during the development and progression of PH.

### 1.22

**Preservation of capillary network of the right ventricle in severe experimental pulmonary hypertension**

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The underlying pathogenesis of right ventricular failure (RVF) due to severe pulmonary hypertension (SPH) remains unknown. Recent studies have proposed that rarefaction of the right ventricle capillary network leads to progressive RV ischemia and failure, particularly in the rat model of VEGF receptor blockade by SU5416 combined with chronic hypoxia (SU+CH), and recently, in the monocrotaline model. However, these studies relied on planimetric analysis of histology, which may lead to biased estimates, rather than systematic, uniform, random sampling (SURS) approaches. Given the overall relevance of hypothesized RV capillary loss in RVF due to SPH, we undertook a stringent stereological approach to address if there is loss of capillaries and/or areas of hypoxia and explore the metabolic RV profile in the RV in rats treated with SU+CH. Rats were injected with the hypoxia probe pimonidazole 1 hour before sacrifice; the RVs of rats (n = 4-6) treated with carboxymethylcellulose (CMC) + normoxia (NX), SU + normoxia (SU), chronic hypoxia + CMC (CH), and SU+CH for three weeks were dissected and investigated by SURS-based stereology. SU+CH rats developed SPH (SPAP: 63 ± 18 mmHg), while moderate PH was detected in CH rats (34 ± 5 mmHg); SU increased PAP (23 ± 5 mmHg) and may increase PH (25 ± 5 mmHg) as previously described. SU-CH rats had marked increase of RV free wall volume when compared with the other groups (ANOVA, P < 0.05); overall, the RV free wall volume correlated with mean PAP (R = 0.873, P < 0.05). The increase in RV free wall volume in SU+CH rats was associated with concordant increases in the absolute volumes of myocytes (myocyte hypertrophy), capillaries, and interstitium. The total RV capillary length was greatest in the SU+CH group, totaling 41.8 ± 19.3 meters (vs. CH: 34 ± 12 m; SU: 29.4 ± 7.2 m; NX: 21 ± 8.6 m), and correlated with PAP (R = 0.46, P < 0.05). Importantly, capillary length to myocyte volume ratio, an indicator of myocyte blood supply, was similar in all four groups (P = NS); the same finding pertained to capillary surface area to myocyte volume ratio. Consistent with the observed preserved RV capillary density in rats with SPH, we observed overall similar hypoxic RV tissue in all four groups, at a mean of 0.7% of the total RV tissue in each group. Overall, these findings indicate there is no reduction of RV capillaries in the SPH rat model. We submit that rather there may be growth of capillaries to maintain normal capillary perfusion to RV myocytes as part of the overall RV expansion due to SPH. RV failure may instead involve biochemical, metabolic, and/or signaling events primarily in the RV myocytes, rather than structural alterations. We speculate that RVF may involve (patho) physiological signaling amenable to immediate interventions that may translate in fast recovery as seen when pulmonary artery pressures are normalized, such as that achieved following lung transplantation. Supported by the ARRA RC1HL108049 and Cardiovascular Research and Education Fund awards (to RMT); Parker B Francis fellowship grant and NHLBI KO8HL105536 (to BG).

### 1.23

**Assessment of pulmonary vascular remodeling in pulmonary arterial hypertension in vivo using 18F-FDG PET imaging**

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Pulmonary arterial hypertension (PAH) is a disease of progressive vascular remodeling. Novel drugs targeted directly at halting or reversing the structural changes in blood vessels have been identified. We explored the utility of positron emission tomography (PET) with 18F-fluorodeoxyglucose (FDG) in the assessment of PAH pathology in the monocrotaline (MCT, 60mg/kg) rat PAH model in response to anti-proliferative therapies (dichloroacetate DCA, 70mg/kg, imatinib, 100 mg/kg and sunitinib, 50 mg/kg/day for two weeks). In vivo imaging was performed using a Siemens Inveon small-animal multimodality PET/CT system. Briefly, rats anaesthetized with isoflurane, and after the completion of the CT scan, were injected with approximately 40 MBq of 18F-FDG through tail vein catheter (>0.5ml). Dynamic emission scans were acquired in list-mode format for 60 min using conventional full-ring, whole-body PET. During PET scanning serial blood samples (20 µl each) were taken via a femoral artery line (eight samples) 1, 2, 3, 5, 10, 15, 30, 45, and 60 min. At the end, animals were sacrificed and tissue samples (lung, kidney, liver) were collected for gamma-counting, as well as snap frozen for biochemical measurement. Left lungs were inflated and fixed with 4% formalin for histology examination. For image analysis, regions of interest (ROI) outlining the lungs were manually drawn on CT images (co-registered with PET data) where lung boundaries were clearly visible. CT-drawn lung ROIs were then transferred to the PET images to obtain whole-lung tissue time-activity curves (TACs). Statistical analysis was performed on SUV data collected for the last 30 min of scanning. Cumulative images over 0 to 60 min were used for kinetic analysis of tracer uptake. Kinetic constants k₁ was estimated by fitting ROI-derived time-activity curves (TACs) into the PatLak plot. Increased lung FDG uptake
was identified two weeks after MCT injection and a further increase in FDG uptake was observed in a group of animals scanned at three and a half weeks after MCT injection. DCA and tyrosine kinase inhibitor treatments reduced FDG uptake. Dynamic analysis of PET imaging revealed an increased kinetic rate constant (Ki) of FDG after MCT injection which was normalized following treatment. These PET data were in agreement with other signs of recovery, such as reduced RV hypertrophy and pulmonary vascular muscularization. This study provides a scientific basis for using FDG-PET in the assessment of pulmonary remodeling and antiproliferative treatments for patients with PAH. Supported by British Heart Foundation and a Grant from Pfizer.

1.24 Extracellular superoxide dismutase modulates NALP3 inflammation in chronic hypoxic mouse models

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The antioxidant enzyme, extracellular superoxide dismutase (EC-SOD), expressed in the lung protects against chronic hypoxia pulmonary hypertension (CHPH). Pulmonary vascular remodeling in the pathogenesis of CPH can be characterized by modulation of the extracellular matrix (ECM) and inflammation, two processes that are linked to oxidative stress in the lung. Studies have shown that the highly reactive nature of reactive oxygen species causes fragmentation of hyaluronan (HA), a key component of the vascular adventitia ECM that is detected in plasma and bronchial lavage fluid of humans with PAH. Fragmented HAs can activate inflammatory responses, particularly induction of the NALP3 inflammasome, leading to activation of caspase-1 and subsequently IL-1β and IL-18 proinflammatory cytokines. Recent data implicates macrophage inflammation in human pulmonary hypertension and CPH, associated with an increase in IL-1β. We hypothesize that loss of EC-SOD augments macrophage activation in CHPH, and tested NALP3 inflammasome activation as a key pathway involved in the processing of IL-1β and IL-18. Mice were exposed to hypobaric hypoxia for up to 35 days. Strains included EC-SOD TG (lung overexpression of EC-SOD), EC-SOD KO (total body knockout), and SOD mimetic treated WT mice. To evaluate macrophage recruitment to the lung, cells were counted from bronchoalveolar lavage fluid and from lung isolated CD11b+ cell single suspensions. To assess HA content, inflamed fixed lung sections were immunostained with HA binding protein. HA protein and RNA were isolated from frozen lungs and cells in culture to quantify NALP3, caspase-1, IL-1β, and IL-18 expression. Hypoxia-induced inflammation was evident by macrophage infiltration to the lung, increased HA content in the ECM, and activation of the NALP3 pathway. Macrophage recruitment to the lungs was attenuated in EC-SOD TG mice and augmented in EC-SOD KO mice, evident by ten-fold higher lung macrophages after 21 days of hypoxic exposure. There was increased HA content upon hypoxic exposure and decreased content in SOD mimetic treated WT mice. To evaluate macrophage recruitment to the lung, macrophage infiltration was measured in the lung, increased HA content in the ECM, and activation of the NALP3 pathway. Macrophage recruitment to the lungs was attenuated in EC-SOD TG mice and augmented in EC-SOD KO mice, evident by ten-fold higher lung macrophages after 21 days of hypoxic exposure. There was increased HA content upon hypoxic exposure and decreased content in SOD mimetic treated WT mice.

We and others have previously shown that nitric oxide (NO) levels are low in patients with idiopathic pulmonary arterial hypertension (IPAH). We have recently demonstrated that IPAH patients also have high levels of hyaluronan (HA) a large glycosaminoglycan found in the extracellular matrix. Since both NO and HA are known to play roles in smooth muscle proliferation and migration, we hypothesized that NO effects on smooth muscle cell proliferation could be mediated by HA. Pulmonary artery smooth muscle cells (PASMCs) isolated from pulmonary arteries from explanted IPAH and control lungs were treated with different amounts of NO and agarose gel HA sizing was performed. PASMCs and human umbilical vein endothelial cells (HUVECs) plated on a coverslip were subjected to a controlled wound by a pipette tip. The effect of different levels of NO on wound closure was evaluated in PASMCs and the effect of different sizes of exogenous HA (4.7, 35, 74, and 2milion kDa) on wound closure was evaluated in HUVECS. PASMCs exposed to the 10 uM NO (NO donor NOC-18) had the fastest wound closure rates [wound closure (arbitrary unit, mean ± SD): 0 uM NO 32.9 ± 3.9, 10μM NO 17.3 ± 2.0, 500 uM NO 63.4 ± 9.8]. Furthermore, agarose gel HA sizing showed that treatment of IPAH PASMCs with low levels of NO (10 uM) resulted in increased small molecular weight HA production (~100-200 kDa) compared to control PASMCs. High levels of NO (500 uM) produced mostly large molecular weight HA (>1510 kDa) in both IPAH and control PASMCs. Wounded HUVECs treated with small size hyaluronan (35 kDa) had increased wound closure compared to HUVECs treated with larger HA [wound closure (arbitrary unit, mean ± SD): 4.7 kDa 0.34 ± 0.04, 35 kDa 0.19 ± 0.09, 74 kDa 0.29 ± 0.01, 2 million kDa 0.40 ± 0.03]. NO affects the size of hyaluronan differently in IPAH patients than in controls. Treatment of IPAH PASMCs with low levels of NO (10 uM) resulted in increased small molecular weight HA. In turn, small HA fragments (35 kDa) increase the rate of wound closure in HUVECS. Thus, the small sizes of HA mediated by NO deficiency in IPAH may play a role in the increase vascular proliferation characteristic of this disease.

1.26 NF-κB dimer activity in pul monary hypertension induced by hypoxia

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Hypoxia plays a vital role in the pathogenesis of pulmonary hypertension (PH), and involves significant upregulation of inflammatory pathways. NF-κB regulates the transcription of genes involved in inflammatory responses, cell growth control, and apoptosis. Relation between NF-κB protein expression, the involvement of specific NF-κB dimers [NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB, and C-Rel], and the pathogenesis of hypoxia-induced PH, remains to be determined. Recently, we showed both prophylactic and therapeutic effects of EC-SOD overexpression in hypoxia-induced PH. However, the exact mechanisms of EC-SOD effects remain unclear. We hypothesize that particular NF-κB dimers are involved in the inflammatory responses associated with hypoxia, and that specific inhibition relevant dimers will be of great value in the treatment of hypoxia-induced PH. Here we examined the activities of NF-κB dimers in primary human pulmonary endothelial cells exposed room air and hypoxiaフィオ3%, for 24 h, in presence and absence of additional recombinant EC-SOD protein. Accumulated EP 1 in the culture medium, an important vasodilator and vasoconstrictor in PH, was measured. Hypoxia induced significant and differential increases in the activity of the five NF-κB dimers. These increases were associated with a significant increase in ET-1 accumulation. In the presence of exogenous recombinant EC-SOD, all NF-κB dimer activities were significantly decreased. However, the amount of decreased activity was dimers specific. The decreased NF-κB activity was also associated with a decrease in ET accumulation. Inhibition of specific NF-κB target dimers may allow us to develop directed therapies that modify the activity of a particular isoform without impairing the function of other NF-κB activities which are vital of other NF-κB activities vital for cell function for cell function.
1.27 Serotonylated fibronectin in pulmonary hypertension

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Serotonin (5-HT) and fibronectin (FN) have been identified to participate in pulmonary hypertension (PH). We previously have reported that FN is post-translationally modified by tissue transglutaminase (TGase) to form serotonylated (s-FN) in pulmonary artery smooth muscle cells and that serotonylation stimulates their proliferation and migration. We hypothesized that s-FN and its binding to TGase are elevated in experimental and human PH. FN isolation, electrophoresis and immunoblotting techniques were used. Serum s-FN/FN levels were elevated in 19 consecutive pulmonary arterial hypertension (PAH) patients compared to 25 controls (0.3 ± 0.18 vs. 0.05 ± 0.07, P < 0.001). While s-FN/FN from explanted lungs of another 19 PAH patients was not statistically significant compared with 9 controls (0.424 ± 0.69 vs. 0.143 ± 0.14, P = 0.24), lungs from 4 PAH patients had high values (1.2-2.5). Lung s-FN/FN was increased in mice and rats with hypoxia-induced PH and in rats with monocrotaline-induced PH. In mice, the increase was detected at one week of hypoxia, preceding the development of PH. Hypoxic rats also had elevated serum s-FN/FN. Enhanced binding of TGase to FN occurred in human PH (0.50 ± 0.51 vs. 0.063 ± 0.11, P = 0.002) in serum samples and s-FN/FN and TGase-bound FN were highly correlated (R² = 0.77). TGase/FN was also increased in experimental PH. The findings are consistent with our previous report that demonstrated a mechanism for intracellular action of 5-HT following its internalization by the 5-HT transporter. They show that increased s-FN and binding of TGase to FN occur in human and experimental PH and support the participation of s-FN in PH. Furthermore, the results offer a potential biomarker for the disease.

1.28 Characterization of the vascular response to acute inflammatory injury in the lung

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Angiogenesis is recognized as a fundamental process in tissue repair after injury however, our knowledge of angiogenesis in the context of lung repair is limited. Specifically, the time course and extent of new blood vessel growth in the lung during normal repair after acute inflammatory injury has not been investigated. This study aims to address these aspects of the vascular response to injury. A greater appreciation for vascular involvement in normal repair may ultimately be important in understanding mechanisms of disease in conditions linked to aberrant wound healing and pulmonary hypertension such as idiopathic pulmonary fibrosis. Eight to ten week old female C57BL/6 mice were treated with bleomycin by intranasal instillation. Lungs tissue was harvested at selected time points after bleomycin administration and enzymatically digested. Immunostaining was performed with antibodies to CD45, CD31, and thrombomodulin for the detection of endothelial cells using flow cytometry. Total lung endothelial cell numbers were calculated. Similar staining techniques and flow cytometry were also done using Tie2-GFP transgenic mice. For the purpose of stereologic assessment, immunohistochemistry was performed on lung tissue sections using an antibody against thrombomodulin to identify pulmonary vasculature. Calculation of vascular surface area is being performed using methods of stereology. Flow cytometry performed on whole lung digests allowed identification and quantification of a discrete endothelial cell population. This method for identification of endothelial cells was verified using Tie2-GFP transgenic mice. Total endothelial cell numbers increased over the course of lung injury peaking at day 21 compared to controls in C57BL/6 mice (P < 0.001) and thereafter trending toward baseline in temporal correlation with pathologic resolution of injury. Lung tissue sections from C57BL/6 are being stained for further stereologic analysis and vascular surface area quantification. Whole lung digestion and flow cytometry provides a reliable, reproducible way of determining endothelial cell number. Our findings suggest there is new blood vessel formation after acute inflammatory injury in the lung followed by blood vessel regression which occurs in parallel with resolution of injury.

1.29 Mitochondrial dysfunction underlies susceptibility of rats with low intrinsic aerobic capacity to hypoxia-induced pulmonary hypertension

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Low aerobic exercise capacity has been linked with a higher probability of premature death in several diseases. Here, we investigated mitochondrial functionality in conferring susceptibility of low exercise capacity rats to pulmonary arterial hypertension (PAH). High (HCR) and low (LCR) exercise capacity rats were exposed to a 10% O2 environment for 21 days. Echocardiographic, hemodynamic, histological were assessed to enable tracking of disease progression.

LCR rats developed significantly greater PAH pathologies compared to HCR with regard to cardiac and pulmonary vessel remodeling, right ventricular pressure (WP) and echocardiographic measures. To determine underlying differences in mitochondrial functionality, we performed genomic analyses of mRNA isolated from the right lung of HCR and LCR exercise capacity rats exposed to either normoxia or hypoxic conditions. Using a pathway-specific array for mitochondrial energy metabolism, marked up-regulation of genes functionally linked to electron transfer within Complex I including Ndufa8 were noted in HCR compared to LCR under normoxia suggesting strain differences in the efficiency of electron transfer. Following hypoxic stimulus, LCR rats demonstrated up-regulation of mitochondrial uncoupling protein 3 (UCP3) and genes associated with Complex IV. By contrast, there was limited alternation of mitochondria-specific genes in HCR under hypoxia conditions implying adaptive responses of LCR to meet energy demands under hypoxic conditions. Taken together, these data support our hypothesis that intrinsically low aerobic capacity may predispose individuals to developing PAH and suggest that impaired mitochondrial function may underlie exacerbation of PAH symptoms.

1.30 Enhanced expression of caveolin01 in smooth muscle cells may determine irreversibility of pulmonary hypertension

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In infants and children, pulmonary hypertension (PH) associated with congenital heart defect and increased pulmonary blood flow, drug toxicity, or respiratory distress syndrome (RDS)/bronchopulmonary dysplasia (BPD) with inflammation show: (1) loss of endothelial caveolin-1 and vWF; and (2) enhanced expression of caveolin-1 in smooth muscle cells (SMC). In an older child, these changes were followed by neo-intima formation and a rapid downhill course. In contrast, in PH associated with RDS/BPD without an accompanying inflammatory disease, there is no loss of endothelial caveolin-1, vWF or enhanced expression of caveolin-1 in SMC. In the monocrotaline (MCT) model, progressive loss of endothelial caveolin-1 and...
concomitant activation of proliferative pathways occur before the onset of PH. Other membrane proteins (PECAM-1, Tie2, sGC) are lost in tandem with caveolin-1, indicating a generalized disruption of endothelial cell membrane. By two and three weeks, cytosolic proteins (HSP90, Akt, 1xβ, α, and eNOS) are lost, but not vWF. At four weeks, the loss of vWF is observed in 29% of the pulmonary arteries, and 70% of these arteries exhibit enhanced expression of caveolin-1 in SMC. We conclude that endothelial caveolin-1 loss or dysfunction may be an initiating factor in the pathogenesis of PH. Endothelial disruption is progressive, and the vWF loss is indicative of extensive endothelial damage and/or loss. Resulting exposure to direct pressure and shear stress may facilitate enhanced expression of caveolin-1 in SMC and its translocation from caveole; ultimately leading to SMC phenotype change, cell migration, and neo-intima formation; and contribute to the irreversibility of the disease. Thus, caveolin-1 has a cell-specific dual role in the context of the disease stage in PH.

1.31 A combination of biomarkers and hemodynamics predicts outcomes in children with pulmonary arterial hypertension

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Although the treatment for severe pulmonary arterial hypertension (PAH) has improved, predictors of survival are poorly described in children with PAH. The aim of this study was to determine, which clinical or blood biomarkers can provide strong independent prognostic information in the management of children with PAH. This single-center, retrospective cohort study was performed using clinical data and blood samples from children with PAH between May 2001 and October 2008 at Children’s Hospital Colorado. In total, there were 92 PAH children with a median age of 8.5 years-old (IQR 4-13.5 years-old) with 46 (50%) female patients. Fourteen children (15.2%) died during follow-up. Mean follow-up time was 3.7 ± 2.4 years, the median was 3.5 years. In total there were 45 variables analyzed, which included 27 protein biomarkers, six clinical markers and 12 hemodynamic. Survival rates from time of sample collection at 1 year, 3 years, and 5 years were 95%, 85% and 91%, respectively. Tissue inhibitor of metalloproteinases-1 (TIMP1) was the best overall predictor of mortality for the biomarkers and clinical variables in the univariate model (c-index = 0.63, P-value = 0.002). Random forests were used to investigate the contribution of clinical, hemodynamic and proteomic predictors of a cause mortality. Seven variables were identified as the most predictive from this analysis: TIMP1, right ventricle to left ventricle systolic pressure ratio, Apolipoprotein A1 (prostacyclin stabilizing factor), age at diagnosis, Uric Acid, height percentile and pulmonary vascular resistance index (in order of the variable importance value). Higher values for TIMP1 and uric acid and lower values for Apo A1 were associated with decreased survival. A combination of biomarkers and clinical variables better predicts survival in pediatric PAH than clinical variables alone.

1.32 Peripheral chemoreceptor responsiveness and hypoxic pulmonary vasoconstriction in humans

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Animal studies demonstrate that interruption of efferent activity from the peripheral chemoreceptors to the lung either by carotid body ablation or vagal nerve sectioning enhances hypoxic pulmonary vasoconstriction (HPV). We hypothesized that persons with high hypoxic ventilatory response (HVR), reflecting greater hypoxic peripheral chemoreceptor sensitivity, should have less HPV. In 15 healthy subjects we measured HVR (L min−1 % SaO2−1) during 15 min of poikilocapnic hypoxia (0.12 FIO2). We then measured HPV using echosonography, estimating pulmonary artery systolic pressures (PASP) while the subjects breathed in random order 0.21, 0.18, 0.15, and 0.12 FIO2 each for periods of 15 min. We recorded a broad range of HVR (0.05-0.3, mean 0.134 L min-1 %SaO2-1). HPV strength was measured as PASP at a common arterial oxygen saturation (SaO2) of 85%. We chose this strategy to obtain a common equal P O2, using SaO2 as a surrogate for aequorin P0, the predominant stimulus to HPV. This yielded a range from 21.7-4.13, mean 28.5 mmHg. We found a significant inverse correlation between poikilocapnic HPV and HPV (P < 0.01; R2 = 0.45). We conclude that there is a direct contribution of the peripheral chemoreceptors to HPV in humans and speculate that the two responses (exaggerated HPV and low HVR) typical of individuals susceptible to high altitude pulmonary edema (HAPE) are linked.

1.33 Gene deletion of JNK1/2 blunts vascular remodeling in hypoxia-induced pulmonary hypertension

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The pathogenic findings in pulmonary hypertension (PH) include marked cell proliferation and structural alterations of the pulmonary arterial (PA) wall that compromise vessel diameter and vasodilator function. Two ubiquitously expressed mitogen-activated protein kinases, c-Jun N-terminal kinase (JNK) 1 and JNK2, are activated in experimental models of PH. However, the contribution of these kinases to the pathogenesis of PH has not been explored. We hypothesized that JNK1/2 promote PA structural remodeling during the progression of hypoxia-induced PH. To test our hypothesis, we exposed adult wild-type (wt) JNK1 null (jnk1 −/−) and JNK2 null (jnk2 −/−) mice for 6 weeks to either ambient air (normoxia) or 10% O2 (hypoxia). Total body weight gain was impeded more by hypoxia in wt mice (77% reduction) compared to jnk1 −/− and jnk2 −/− mice (36% and 32% reduction, respectively). In contrast, right ventricular systolic pressure and the right ventricle/left ventricle + septum ratio were similarly elevated by hypoxia in all three genotypes. Hypoxia for six weeks induced a marked increase in collagen deposition in wt lungs, but not in the lungs of hypoxic jnk1-/- or jnk2-/- mice. Preliminary morphometric analysis of small pulmonary arteries (50 – 100 mm in diameter) revealed hypoxia-induced remodeling of the vascular wall in wt lungs, which was evident as wall thickening. This abnormality was not observed in lungs of hypoxic jnk1-/- or jnk2-/- mice. Collectively, our findings suggest that jnk1-/- or jnk2-/- gene deletion partially prevents hypoxia-induced weight loss, collagen deposition and small vessel wall thickening. Association of these findings with improved pulmonary vascular function would provide a basis for considering JNK1 and JNK2 as potential molecular targets for therapeutic intervention in PH.

1.34 Microparticles from mice with monocrotaline-induced pulmonary hypertension induce right ventricular hypertrophy and pulmonary vascular remodeling in healthy mice

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We have shown that murine lung-derived microparticles (MPs) alter narrow cell phenotype by inducing expression of lung-specific mRNA and protein. We hypothesized that lung and/or plasma-derived MPs (LMPs, PMPs) play a role in the pathogenesis of pulmonary arterial hyper-tension by inducing angioproliferative changes in pulmonary vascular endothelial or bone

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Abstracts

1.35 IL-13, IL-17 and B cell response in pulmonary hypertension

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Immune responses have long been associated with pulmonary arterial hypertension. Previous studies in our lab have shown that a prolonged TH2 response results in elevated anti-angiogenic factors that induce severe pulmonary arterial remodeling. In the present study, we investigated the role of the immune response (B cells, Interleukin-13, IL-13, IL-17) in pulmonary hypertension induced by antigen and urban PM. The respiratory fraction of airborne PM was collected in New York City. Th2 primed mice were challenged with soluble antigen (ovalbumin) combined with urban PM2.5 intranasally. We determined pulmonary arterial remodeling by histology, right heart hypertrophy by ventricular weight measurements and right ventricular systolic pressures recorded in anesthetized mice that spontaneously breathe room air. In contrast to wild type, mice deficient in B cells had no significantly increased right heart weights, or right heart systolic pressures in response to intranasal antigen and urban PM. Reconstitution of antigen-specific antibody restored the development of pulmonary hypertension in these mice. Combined blockade of IL-13 and IL-17 significantly ameliorated pulmonary hypertension induced in wild type mice exposed to antigen and urban PM2.5. Our studies indicate that antigen-specific antibody, IL-13 and IL-17 are necessary for the development of pulmonary hypertension induced by the exposure to antigen and urban PM2.5.}

1.36 Exercise training in pulmonary arterial hypertension associated with connective tissue diseases

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The objective of this prospective study was to assess short- and long-term efficacy of exercise training (ET) as add-on to medical therapy in patients with connective tissue diseases-associated pulmonary arterial hypertension (CTD-APAH). Patients with invasively confirmed CTD-APAH received ET in-hospital for three weeks and continued at home for 15 weeks. Efficacy parameters have been evaluated at baseline and after 15 weeks by blinded-observers. Survival rate has been evaluated in a follow-up period of 2.9 ± 1.9 years. Twenty-one consecutive patients were included and assessed at baseline, and after 3 weeks, 12 after 15 weeks. Patients significantly improved the mean distance walked in six minutes compared to baseline by 67 ± 52 meters after three weeks (P < 0.001) and by 71 ± 35 meters after 15 weeks (P = 0.003), scores of quality of life (P < 0.05), peak oxygen consumption, oxygen saturation and maximal workload. Systolic pulmonary artery pressure and diastolic systemic blood pressure improved significantly after three weeks of ET. The 1- and 2-year overall-survival rates were 100%, the 3-year survival 73%. In one patient lung transplantation was performed six months after ET. ET as add-on to medical therapy is highly effective in patients with CTD-APAH to improve work capacity, quality of life and further prognostic relevant parameters and possibly improves the 1-, 2- and 3-year survival rate. Further randomized controlled studies are needed to confirm these results.

1.37 Inhibition of Gβγ signaling decreases pulmonary artery pressure and regresses vascular remodeling in experimental pulmonary hypertension

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G proteins are involved in signaling and proliferation of vascular smooth muscle cells. G proteins mediate cellular signaling through their subunits, Ga and Gβγ. Inhibition of Gβγ signaling inhibits intimal hyperplasia in vein grafts and carotid artery injury. However, the relevance of Gβγ signaling to the vasculature in pulmonary hypertension (PH) is unknown. We hypothesized that Gallein, an inhibitor of Gβγ-binding, would reduce PH in monocrotaline (MCT) and Sugen 5416 (SU5416/Hypoxia PH models, by inhibiting the hypertrophy-inducing G protein mechanism. Flow cytometry was performed to determine the effect of Gallein on pulmonary artery smooth muscle cell (PASMC) proliferation. Western blot was performed to determine the effects of Gallein on ERK activation, as measured by phosphorylation and normalized to total ERK protein expression. Adult male rats received MCT (60 mg/kg) and were treated with Gallein 15 mM (0.1 ml/day sc ×3 week, then 0.3 ml/day × 1 week and 0.5 ml/day × 1 week, n = 10) or equivalent saline (vehicle, n = 9). SU5416/Hypoxia rats (n = 6) were also treated with this protocol. Hemodynamics were assessed by echocardiography and intimal hyperplasia. Lung histology was performed and Gallein caused a dose-dependent inhibition of proliferation in PASMC from MCT rats (%Gallein decreased to 3% and 1.5% at 10 and 1 M Gallein, respectively). Gallein (20 µM) decreased proliferation in PAH human PASM Cs. Gallein also decreased ERK activation in MCT rats PASM Cs. Gallein caused a significant decrease in mPAP in MCT-PH (32 ± 1.7 vs. 42 ± 2.3 mm Hg, Gallein vs. placebo, respectively P = 0.015) and increased treadmill walking distance (125 m vs. 36 m, P < 0.05). Gallein decrease % medial thickness of small pulmonary arteries (24 ± 2.3 vs. 32 ± 1.1 %, P = 0.019) in MCT rats. Gallein also increased the cardiac index in SU5416/Hypoxia rats (0.38 vs. 0.28 ml/min/g), although the pulmonary artery acceleration time did not prolong (21 ± 1.7 vs. 19 ml ± 2 ms, P = 0.3). Inhibition of G protein receptor kinases reduces PASMC proliferation in human and experimental PAH. Inhibition of Gβγ signaling reduces PH in experimental models and holds promise as a therapeutic strategy in PH.

1.38 Alterations of pulmonary artery metabolism in severe pulmonary arterial hypertension

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Severe pulmonary arterial hypertension (SPH) is characterized by extensive...
remodeling of pulmonary arteries, which has become that main target of established therapies involving prostacyclin, phosphodiesterase 5 inhibitors, and endothelin receptor blockers. In a pathological comparison between 62 PAH and 28 normal explanted lungs collected by the pulmonary hypertension breakthrough initiative (PHBI), we found that total pulmonary artery wall and intima thickness were significantly increased in PAH lungs and correlated with pulmonary hemodynamics. These findings pertained to patients that were treated with the current state of the art medications for PAH listed above, suggesting that despite their beneficial clinical effects; these therapies may have limited impact on pulmonary vascular remodeling in PAH patients. An emerging alternative concept is that the metabolic adaptation of pulmonary vascular cell plays a key role in pulmonary vascular remodeling in PH. We showed that these metabolic alterations would involve upregulation of HIF-1α and therefore favor the preferential use of glycolysis for vascular cell growth that underlies the disease. We therefore addressed the pattern of expression of key genes that code for the expression several enzymatic steps in glycolytic, fatty acid and mitochondrial metabolism in PAH and normal pulmonary arteries. OCT-embedded frozen IPAH, collagen vascular disease-associated associated PAH (APAH), and normal lung samples (N = 6 each) were obtained from explanted lungs accrued by the PHBI. Enrichment for pulmonary vessels, including vascular lesions (in PAH lungs), were achieved by selective structure harvesting with laser capture microdissection. RNA was then extracted, reverse transcribed, and the resultant cDNA amplified using the profiler PCR Arrays (SA Biosciences), aimed at the quantifying mRNA expression of members of the fatty acid, glucose, and mitochondrial energy metabolism pathways. Two-fold or higher differences in gene expression (versus controls) were considered significant [with a P < 0.05]. No significant differences were seen in the glycolytic and mitochondria genes between IPAH versus controls; however, APAH lungs had increased expression of several enzymes, including hexokinase 2 (4.1-fold) and pyruvate dehydrogenase kinase 2 (4.4-fold); the expression of aconitase 1 and glucose-6 phosphate dehydrogenase isoform 1 was increased when comparing the IPAH to APAH lungs. APAH lungs had increased expression of 64 members of the mitochondria metabolic enzymes compared to control and IPAH, suggesting significant upregulation of mitochondria metabolism in APAH. IPAH lungs however showed a significant upregulation of 28 genes encoding for fatty acid metabolism versus control, with only 13 fatty acid metabolism genes upregulated in APAH lungs versus controls, and no significant differences in PAH and APAH in fatty acid metabolism enzymes. Protein and enzymatic activities of candidate genes involved in vascular cell proliferation are being carried out and will be presented. Our study provides the first comprehensive assessment of gene expression of enzymes involved in key vascular cellular metabolic processes in PAH. These processes regulate several key cellular features, including cell growth and resistance to apoptosis, which have been shown to play central roles in the pathogenesis of the disease. Our study, reliance on diseased and control human samples is novel, and will provide a roadmap for mechanistic studies in relevant animal models of severe pulmonary hypertension. Supported by the ARRA RC1HL100849 and Cardiovascular Research and Education Fund awards (to RMT); Parker B Francis fellowship grant and NHLBI RO8HL105536 (to BG).

1.39 Mitofusion-2 plays a critical role in mitochondrial dysfunction in pulmonary hypertension and is a potential therapeutic target

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Excess cell proliferation in pulmonary arterial hypertension (PAH) is favored by excessive fission and impaired fusion of mitochondria in pulmonary artery smooth muscle cells (PASMCs). We hypothesize that excessive mitochondrial fragmentation in PAH, mediated in part by a deficiency of mitofusin-2 (MFN2), permits excessive PASMC proliferation. We further assessed whether restoring MFN2 expression is therapeutic. MFN2 mRNA levels were measured in human PAH PASMCs and from lungs of monocrotaline (MCT) and Sugen 5416 (SUS416) / Hypoxia rat models of pulmonary hypertension (n = 4–10). Western blots for MFN2 were performed on human PAH PASMCs (n = 4). PASMCs were infected with rat MFN2 (Ad-MFN2) or green fluorescent protein (Ad-GFP) to assess the effects of augmenting MFN2 on proliferation (measured using EdU labeling) and apoptosis (determined by Annexin-V flow cytometry). Female SUS416/Hypoxia rats were nebulized with Ad-MFN2 or Ad-GFP to assess the effects of augmenting MFN2 in vivo. Quantitative lung histology was used to assess vascular remodeling in the lungs of SUS416/Hypoxia rats. MFN2 levels are decreased in human PAH PASMCs and in lungs of MCT and SUS416/Hypoxia rats (relative reduction of 20% MFN2 mRNA expression compared to controls). Administration of MFN2-kibed adenosine to MCT rat PASMCs significantly decreased proliferation versus control adenovirus (2.4% vs. 29%, P < 0.05) and also increased the number of apoptotic cells (2.5% vs. 1.5%, P < 0.05). Ad-MFN2 also tended to decrease medial thickness of small PAs (21% vs. 24%, P = 0.29) in SUS416/Hypoxia rats. In SUS416/Hypoxia rats, treatment with Ad-MFN2 vs. Ad-GFP tended to increase cardiac output (85 ml/min vs. 75 ml/min, P = 0.18), treadmill walking distance (370 m vs. 324 m, P = 0.056). MFN2 levels are decreased in human PAH and in two rodent models of PAH. Augmentation of MFN2 levels decreases proliferation and enhances apoptosis in PASMCs and tends to improve hemodynamics in experimental PH. Further research is required to determine the therapeutic potential of Ad-MFN2 as an antiproliferative, proapoptotic therapy in PH.

1.40 Macrophage migration inhibitory factor deficiency promotes increased right ventricular endostatin expression and reduced capillary density in a model of chronic hypoxic pulmonary hypertension

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Right ventricular (RV) remodeling is a critical compensatory mechanism in chronic pulmonary hypertension (PH), though little is known about mechanisms of RV microvascular remodeling. We have observed increased RV expression of macrophage migration inhibitory factor (MIF), an inflammatory mediator with proangiogenic effects, in mice exposed to chronic hypoxia (CH). Recently, we observed a negative correlation between circulating levels of MIF and endostatin, a potent angiostatic factor, in serum from PH patients. We hypothesized that MIF may modulate endostatin expression in the RV, potentially altering CH-induced RV angiogenesis. PH was induced in wild type (MIF+/+) and MIF-null C57 BL/6 mice exposed to CH (10% O2). RV capillary density was assessed by histochromic staining. MIF and endostatin expression were measured by western blot. Expression of the endostatin precursor collagen 18A1 (Col18A1) was measured by quantitative PCR. In separate experiments, an immortalized, cardiac myocyte-derived cell line (HL-1) was exposed to hypoxia after suppression of MIF expression with RNA interference (RNAi). After six weeks of CH, RV mass, myocyte cross-sectional area, and capillary density increased significantly in MIF+/+ mice coincident with a 3.1-fold increase in RV MIF protein. In contrast, RV endostatin expression doubled, and CH-induced increases in both capillary density and myocyte area were blunted in MIF-null mice. RV Col18A1 expression was increased by CH in both mouse strains, but CH-associated increases in RV endostatin protein were only observed in MIF-null mice. Finally, RNAi-mediated MIF suppression in HL-1 cells was sufficient to enhance endostatin protein expression in vitro. These findings suggest that MIF may inhibit myocardial endostatin expression. Furthermore, increased endostatin expression is associated with reduced RV angiogenesis in a mouse model of CH-PH implicating MIF as a novel determinant of RV angiogenesis/vasclogenesis.

1.41 Premature differentiation of vascular smooth muscle cells in human congenital diaphragm hernia

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Pulmonary hypertension is a major determinant of mortality and morbidity in congenital diaphragmatic hernia (CDH), which is associated with characteristic thickening of the media and adventitia of the vascular wall. The media consists of a heterogeneous population of smooth muscle cells (SMC), ranging form synthetic to contractile cells, which is associated with their regulatory (vascular tone) and structural functions. These populations are regulated by developmental and environmental cues and thus may play a role in the development of the structural changes. We first analyzed the protein expression of specific markers associated with either synthetic or contractile SMC phenotypes in human lungs at different developmental stages and secondly, we compared immature/premature and term infants with congenital diaphragmatic hernia or lung hypoplasia due to renal agenesis or PROM with age-matched control infants. Synthetic and contractile SMCs are distributed in a temporal and spatial specific pattern, associated with the proximodistal axis of the lung. Infants with CDH have contractile SMCs which are more widely distributed and already more distally located compared to controls. This different distribution is already observed form 19 weeks gestation onwards. For the first time it is shown that the more extensive distribution of contractile SMCs is associated with an early maturation of the vasculature, contrasting to the general hold concept that the lung shows delayed maturation in CDH.

1.42
The development of pulmonary hypertension after first episode of acute pulmonary embolism and related risk factors

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Most patients with acute pulmonary embolism (PE) will have a good prognosis if they received proper treatment. However, part of them will develop into pulmonary hypertension (PH) including chronic pulmonary thromboembolic hypertension (CTEPH). Risk factors related to the development of PH after acute PE need to be addressed to guide the clinical practice. Consecutive patients diagnosed as first episode acute PE admitted to the institute from January 1, 2006 to January 31, 2010 were included in this study. All the patients included were followed up in outpatient department or through telephone by at least two of the investigators. Their symptoms, physical signs and medical examination results were recorded. During the follow-up period, patients with PH showed in ultrasonogram (UG), defined as estimated systolic pulmonary artery pressure (sPAP) over 50 mmHg, and received more precise examination like digital subtraction pulmonary angiography and Right heart catheterization to confirm the diagnosis of CTEPH. From 2006 to 2010, 675 patients diagnosed as acute PE were screened and 612 patients of them were included in this study. After a median follow-up period of 36 months, all-cause mortality was 17.3%. Fifteen patients developed into PH showed in UG and 10 of them were diagnosed as CTEPH. The cumulative incidence of PH after acute PE was 1.0% (95%CI 0.2%-1.8%) at one year, 1.3% (95%CI 0.3%-2.3%) at two years and 3.5% (95%CI 1.5%-5.5%) at five years. Patients with history of varicose vein of lower limbs (HR 5.480, 95%CI 1.058-23.835) and those with existence of PH at the first episode of PE (HR 10.743, 95%CI 2.315-49.852) had higher risk in developing into PH. PH is one step in the natural history of acute PE. The cumulative incidence of PH after acute PE was 3.5% at five years. History of varicose vein of lower limbs and high estimated pulmonary artery systolic pressure over 50 mmHg at the first episode of PE seem to increase the risk of CTEPH.

1.43
Activation of NRF2 attenuates hypoxia-induced cardiopulmonary alterations in mice

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NF-E2-related factor 2 (Nrf2) is a key transcription factor that inductively activates a battery of antioxidative enzymes in various tissues in response to an increase in oxidative stress. It has been reported that oxidative stress accumulates in the lungs of patients with severe pulmonary hypertension (PH), and this stress likely plays a crucial role in their cardiopulmonary changes. We hypothesized that activation of Nrf2 would show therapeutic efficacy against such cardiopulmonary changes in a hypoxia-induced PH model. To test this hypothesis, wild-type (WT) and Nrf2-deficient mice, as well as Kelch-like ECH associated protein 1 (Keap1, a main actor in Nrf2 degradation pathway which inhibits Nrf2 activity)-knockdown mutant mice were exposed to hypobaric hypoxia for 3 weeks. Subsequently, right ventricular systolic pressure (RVSP), right ventricular hypertrophy (RVH), and pulmonary vascular remodeling were assessed. Hypoxic exposure exacerbated RVSP, RVH, and pulmonary vascular remodeling in WT mice. However, interestingly, simultaneous administration of oltipraz, a potent Nrf2 activator, significantly attenuated RVH and pulmonary vascular remodeling in WT mice. As anticipated, the hypoxia-exposed Nrf2-deficient mice developed more pronounced RVH than the WT mice. Furthermore, oltipraz treatment showed no therapeutic effect in the hypoxia-exposed Nrf2-deficient mice, indicating that the therapeutic effect of oltipraz on cardiopulmonary changes is dependent on Nrf2 activity. Hypoxia-exposed Keap1-knockdown mice showed less RVH and pulmonary vascular remodeling than WT mice, underscoring the beneficial potency of constitutive Nrf2 activation. In conclusion, both pharmacological and genetic activation of Nrf2 decreased RVH and pulmonary vascular remodeling in a murine model of hypoxic PH. The therapeutic efficacy of oltipraz highlights the promising therapeutic potency of Nrf2 activators for the prevention of PH.

1.44
Pulmonary artery vortex parameters for the prediction of pulmonary vascular hemodynamics

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Four dimensional flow cardiac magnetic resonance imaging (4D CMR) allows for qualitative and quantitative assessment of complex blood flow in the proximal pulmonary arteries. 4D CMR analysis of main pulmonary artery (MPA) flow in pulmonary hypertension (PH) subjects has demonstrated a single systolic vortex blood flow formation that is absent in controls (Reiter, et al., Circulation 2008). Using 4D CMR in both PH subjects and healthy controls, we sought to 1) characterize MPA complex blood formations, and 2) determine if MPA vortex parameters predict pulmonary vascular hemodynamics. As part of a prospective IRB-approved study, seven subjects with right heart catheterization-proven PH and five age-matched controls underwent CMR acquisition. MPA diameter (MPAD), right ventricular end diastolic volume (RVEDV), end systolic volume (RVESV), ejection fraction (RVEF), and cardiac output (CO) were calculated using the Siemens Argus application. 4D CMR flow visualization was performed using the Siemens 4D Flow Viewer. All subjects underwent same-day 2D and Doppler echocardiography for assessment of 1) right ventricular diastolic function including tricuspid valve (TV) E, A, e’, and a’ velocities, as well as E/A and E/’e’; and 2) right ventricular systolic pressure (RVSP). 4D viewer analysis revealed MPA systolic vortex formations in six of seven PH subjects, while all five healthy subjects had unidirectional non-vortical MPA flow. Four PH subjects demonstrated two mirror image simultaneous MPA vortex formations, while the other two PH subjects had only one vortex. RVSP in PH subjects was significantly elevated vs. controls (54±4-21 mmHg vs. 24±4-10 mmHg, P=.004). Using multiple regression analysis, the number of MPA vortices positively correlated with RVSP (P=.06, p<01), RVEDV (P=.05, p<02), MPAD (P=.1, P<01), CO (P=.06, p<02), and TV E/’e’ (P=.79, p<0.002) and negatively correlated with RVH (P=.04, p<0.02).
correlated with e' (±6.6, p<0.02) and E/A (±69, p<0.01). No statistically significant relationship existed between MPA vortex number and RV/EF, RVESV, and TV a'. In contrast to previous work, we describe the presence of multiple MPA vortex formations in a small cohort of PH subjects. The number of MPA vortices correlated with markers of disease severity including pulmonary pressure, RV diastolic function, and ventricular remodeling. Further characterization and quantification of MPA flow patterns may reveal novel insights into right ventricular-pulmonary arterial coupling, biomechanics, and bioenergetics.

1.45 Endothelial Krüppel-like factor 4 modulates pulmonary arterial hypertension

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Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor expressed in the vascular endothelium, where it promotes an anti-inflammatory, anticoagulant, and anti-proliferative phenotype, and induces the expression of endothelial nitric oxide synthase (eNOS). Microarray data from two independent studies have shown that KLF4 expression is decreased in human lung tissues from patients with PAH. We hypothesize that KLF4 deficiency predisposes to PAH. We utilized a mouse model of PAH wherein pulmonary hypertension is induced by three week exposure of mice to 10% FiO2. Endothelial KLF4 knockdown (KD) was achieved using VE-cadherin Cre x floxed KLF4 mice. After hypoxia treatment, right ventricular systolic pressure (RVSP) and pulmonary arterial pressure (mPAP) were measured via catheterization. Right ventricular hypertrophy was assessed by weighing the right ventricle (RV) and the left ventricle plus septum (LV+S). Lung tissue homogenates were assessed for gene expression using QPCR analysis. Group comparisons were done by ANOVA.

1.47 Pulmonary vasculature develops from WNT2+ cardiac mesoderm coordinated by endoderm-secreted SHH

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The closely coordinated development of the heart and the lung is uniquely illustrated by the formation of a common cardiopulmonary circulation. Despite the importance of this vascular bed, little is known about the origin of the pulmonary vascular progenitors, or the signals coordinating its development. Our lab has previously shown that lung development requires paracrine WNT2 signaling from the cardiac inflow tract. Using inducible-cell lineage tracing, we demonstrate that WNT2+ progenitors surrounding the inflow tract contribute to the pulmonary vasculature in a temporally-restricted manner. WNT2+ mesoderm present at the time of lung initiation gives rise to the adventitial, medial, and intimal layers of the pulmonary vasculature and the cardiac inflow tract. The vascular specification of WNT2+ progenitors occurs early in lung development, as WNT2+ cells induced later fail to contribute to the vasculature. We also demonstrate that Shh derived from the foregut endoderm is required for the emergence of the endothelial plexus that connects the cardiac outflow and inflow tract within the primordial lung mesenchyme. Inducible cell lineage tracing of Shh-receiving cells at the time of lung initiation demonstrate overlap with WNT2+ cell fate, suggesting a functional link between Shh signaling and WNT2+ vascular progenitors. Understanding the role of endoderm-secreted Shh in coordinating the development of WNT2+ mesoderm would provide critical insights about the nature of epithelial-mesenchymal interaction in pulmonary vascular formation and remodeling.

1.48 A female model of severe neointimal pulmonary hypertension: Evidence for increased susceptibility in a female rat following pneumonectomy and monocrotaline

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Endothelium is of essential importance for the maintenance of vessel function. Endothelial dysfunction is considered as a key event in the pathology of cardiovascular diseases. Newer studies have proven that progressive ischemic dysfunction and organ damage can be prevented and restored by new therapeutic strategies like cell-based therapy. These attempts encompass application of autologous progenitor cells derived from peripheral blood or bone marrow. The accurate definition and origin of progenitor cells and their function during vessel recovery still remain as unsolved issues. Peripheral blood contains only a low fraction of endothelial progenitor cells and extraction of these cells from bone marrow is based on invasive methods. Embryonic stem (ES) cells can develop into any functional cell type, and may serve as a source for cell therapy. Nevertheless, because of ethical concerns about and immunogenicity of ES cells, their clinical use is still controversial. These problems can be avoided by application of induced pluripotent stem cells. The present work focuses on development of a method to routinely derive large numbers of endothelial cells from differentiating murine ES cells. Transgenic cell lines expressing a reporter and a resistance gene under the control of endothelial-specific promoters such as Flk-1 and VE-Cadherin were established, allowing for antibiotic selection of desired cells as a solution to the problems linked with mechanical isolation. Gene transfer in ES cells was carried out by lentiviral transduction, followed by antibiotic selection of stably transfected clones. Taking advantage of this approach yielded robustly positive results with a very high efficiency, leading to the establishment of several ES cell clones, expressing GFP and zeocon under the control of the Flk-1 and VE-Cadherin promoter, respectively. The generated ES cell lines will later allow for the antibiotic-supported isolation of Flk-1 or VE-Cadherin positive cells. In addition, the cell-specific GFP expression in these cell lines will also be useful for other applications such as enhancement of differentiation towards endothelial cells, as well as developmental studies.
Decades of important animal studies in pulmonary hypertension have utilized male rats, exposed either to chronic hypoxia or monocrotaline (MCT), to define disease mechanisms and test novel treatment strategies. However, females represent 70-80% of the afflicted human population, and a female rat model with the key features of human disease would be useful for testing hypotheses about sex and the susceptibility to PAH. Unfortunately, previous studies exposing female rats to MCT or hypoxia did not demonstrate significant pulmonary hypertension. We sought to establish a female rat model of PAH with neointimal formation and right ventricular (RV) failure. Seven days after left pneumonectomy, we administered 40-60 mg/kg of MCT to young rats (male or female); early signs of RV dysfunction were present by Day 10 after MCT using a VisuSoNics 2100 echo. In some animals, echo measures were also made at Day 21 after MCT before the rats were sacrificed for lung micro-CT and histology; other rats were allowed to progress to death. At 60 mg/kg, female rats experienced mortality at least as severe as males, perhaps worse. In contrast to previous findings, micro-CT illustrated more severe vascular pruning in female rats receiving 40 mg/kg MCT as compared to male rats receiving 50 mg/kg suggesting that female rats in this model are more sensitive to MCT. Male and female rats had severe RV dilatation and loss of fractional shortening at Day 21 after MCT. There was no apparent renal or liver disease in males or females as assayed by urine, blood chemistry, and tissue histology on Days 7, 14, and 21 after MCT. We are currently analyzing lung tissue to assess mean linear intercept for loss of alveolar surface. We will also present exciting quantitative data from the micro CT which details length, radius, and branching of the individual vessels in the micro CT images. Finally, we are profiling the MCT metabolites in female and male rats to confirm earlier work demonstrating that female lungs are exposed to less of the toxic metabolite MCT-pyrole. Young female rats treated with relatively low dose MCT following left pneumonectomy develop a severe, neointimal pulmonary vasculopathy with vascular pruning, proliferative lesions, and RV failure. This model appears to offer a unique opportunity to explore hormonal or sex chromosomal influences on the susceptibility to PAH. It also affords the opportunity to examine sex-specific differences in the response to an experimental PAH therapy and the potential to analyze sex-specific RV adaptation to increased afterload.

1.49 Stem cell-like cells in angioproliferative lesions in the SU5416/chronic hypoxia model of angioproliferative PAH

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It is unclear whether stem cells contribute to the angioproliferative obliteration of the pulmonary arteries in IPAH. The goal of our study was to investigate the potential contribution of progenitor and stem cells to the angioblastic process in the SU5416/chronic hypoxia (SuHx) model of severe PAH. We used a) sequential labeling with halogenated thymidine analogues to assess asymmetrical cell division in SuHx animals, and b) treatment with the CXCR4 inhibitor AMD3100 to investigate CXCR4-dependent cell homing to the lungs of SuHx animals with severe angioproliferative PAH. We detected stem cell-like cells in the angioproliferative lesions of SuHx lungs using the following criteria: (1) Positive staining for accepted markers of stem cells; (2) Asymmetric cell division in the lesion cells; and (3) Evidence for multipotency based on immunostaining for markers that characterize adipoid differentiation within the lesions. Preventive treatment with AMD3100 reduced mainly the obliteration of medium sized pulmonary arteries, associated with a moderate decrease in right ventricular systolic pressure (10 mmHg), supporting the concept of site-specific lung vessel remodeling. We have found evidence for the presence of stem cell-like cells within angioproliferative lesions in the SuHx model. Because inhibition of the homing of circulating cells by AMD3100 did only moderately prevent angioblastation, we postulate that stem cells within a pulmonary vascular niche also participate in angioproliferative remodeling in PAH.

1.50 Acute vasodilator testing with sildenafil versus nitric oxide in patients with pulmonary arterial hypertension

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Vasoreactivity testing with inhaled nitric oxide (iNO) is recommended in patients with pulmonary arterial hypertension (PAH) because it predicts the long-term response to calcium channel blockers and prognosis. The PDE-5-inhibitor sildenafil has recently become available as an intravenous formulation. It is tempting to use sildenafil for acute vasoreactivity testing since it is cheaper, more stable and easier to handle than inhaled nitric oxide. But to date, it is not known if the acute responses to sildenafil and iNO are equal within an individual patient. The aim of this study is to compare acute vasoreactivity in response to oral sildenafil vs. iNO in patients with PAH. In this retrospective, open-label, and single center study we included all patients who were admitted to our adult pulmonary hypertension unit from January 2002 to October 2011, met the criteria for PAH, and underwent vasoreactivity testing with iNO and oral sildenafil. Positive vasoreactivity was defined according to the current guidelines. One hundred ninety-eight patients were included. Nine point six percent of the patients met the responder criteria for iNO and 13.6% for sildenafil. iNO responder rate was 10.1% in idiopathic PAH (IPAHC), and 6.1% in associated PAH (PAAH). Eleven point one percent of patients with iPAH and 12.1% of patients with APAH were acute sildenafil responders. Intra-individually, the NO-induced decrease in mPAP correlated with the decrease in mPAP after sildenafil administration (r = 0.52, P < 0.001). The same was true for the increase in CO in response to both drug (r = 0.51, P < 0.001). Applying the current response criteria, the sensitivity to detect iNO-responders by sildenafil vasoreactivity was 81.3%, the specificity was 94%, the positive predictive value was 48%. In PAH patients the vasoreactive response to sildenafil is stronger than to iNO. The intra-individual vasoreactive responses to both drugs correlate. The sensitivity to detect NO-responders by using sildenafil for vasoreactivity testing was moderate, but the positive predictive value was low.

1.51 Ambrisentan for therapy of portopulmonary hypertension (POPH): Update on safety and efficacy

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To present an update of the long-term hemodynamic response and clinical outcomes of POPH patients treated with ambrisentan at Mayo Clinic. Observational study of prospectively identified POPH patients from 01/2007 to 12/2011 treated with ambrisentan. Clinical data, baseline and follow up transthoracic echocardiograms (TTE) and right heart catheterizations (RHC) were accomplished and compared. A total of 27 patients with POPH were started on ambrisentan therapy. Median age (IQR) of the cohort was 56 (53-60). Fifteen were female (55%) and 25 were Caucasians (93%). Patients were followed for a median of 874 days (472-1548). Median time on ambrisentan therapy was 391 days (259-839). Ten patients have undergone liver transplantation successfully. Nine out of 27 patients died, 7 deaths were attributed to complications of chronic liver disease, one patient died of sepsis, and one patient died of an acute coronary syndrome. Follow up RHC data were available in 20 patients and was performed at a median of 416 days (254-838) after the initial diagnosis. Mean pulmonary artery pressure (mPAP) improved from 42 mmHg (35-57) to 38.5 (28-43.5), P = 0.001; pulmonary vascular resistance (PVR) improved from 434 dynes·s·cm⁻⁵ (311-611) to 228 (154-361), P = 0.001, and cardiac output increased from 6 L/min (5.7-4) to 7.9 (6.4-9.2), P = 0.005. Ten patients normalized their PVR after initiation of therapy. TTE data available in all patients showed that RV size and function improved in 19 and 18 patients respectively (function normalized in 16). Ambrisentan was
well tolerated in all but one patient who developed severe edema and required discontinuation in less than two weeks of initiation. No significant elevation of transaminases requiring discontinuation of the medication was identified. In this prospective cohort of patients with POPH, ambrisentan proved to be safe and efficacious for the therapy of POPH patients. Ambrisentan resulted in significant improvement in hemodynamics and normalization of RV size, function and PVR in the majority of the patients.

1.52 Right ventricular dysfunction due to pulmonary arterial hypertension is characterized by metabolic gene remodeling and abnormal mitochondrial function and maintenance

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Patients with pulmonary arterial hypertension (PAH) die of right ventricular (RV) failure (RVF). Even after successful vasodilator therapy has been established, RV ejection fraction remains the most important survival prognostic factor in patients with PAH. Given the prognostic importance of RV function, the cellular and molecular mechanisms of RVF are of great interest. Abnormal energy substrate utilization has been implicated in the development of chronic left heart failure, but data on metabolic remodeling during RVF remain incomplete. We have characterized metabolic gene expression changes in the hypertrophied non-failing and failing RV, in the setting of chronic pressure overload in two different animal models: SUGA I6/hypoxia (RVF) and pulmonary artery banding (PAB, a model of non-failing RV hypertrophy). Failing RV-tissue exhibits a multilevel impairment of FAO oxidation as evidenced by decreased expression of FAO-related genes encoding key enzymes such as ACADM, ACADVL, ACSL1, CD36, PPAR-α and PGC-1α, the master regulator of FAO and mitochondrial biogenesis in the heart. These changes seem to be independent of chronic pressure overload. To what extent impaired FAO is functionally important in the development of RVF remains to be investigated. Similar to what has been reported, we found that RVF exhibits an increased expression of glycolysis-related genes. Altogether, there is evidence to suggest that RVF is associated with abnormal energy substrate utilization; however, to what extent the mitochondrial biology is impaired, remains unknown. We demonstrated that failing RV-tissue has lower number of mitochondria per gram of RV tissue; these mitochondria have abnormal shape and size and seem to be organized in clusters. Respiratory analysis of isolated RVF-mitochondria revealed decreased mitochondrial respiration, as evidenced by decreased ADP-stimulated (State 3) respiration rate, as well as decreased ADP/O ratio, which measures respiratory efficiency. The structural and functional changes where associated with decreased expression of the mitochondrial transcription factor α (TFαm), which coordinates mitochondrial replication and maintenance. Finally, by a mechanism that is yet to be determined, treatment with carvedilol reverses the metabolic gene remodeling, restores PGC-1α and TFαm expression and normalizes mitochondrial respiration.

1.53 Urokinase plasminogen activator receptor expression in pulmonary venous hypertension due to left heart failure

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Pulmonary hypertension (PH) due to left heart dysfunction also referred to as pulmonary venous hypertension (PVH), is the most common cause of PH in the United States. The presence of PH in conjunction with left heart disease carries a significantly worse prognosis than left heart disease alone; yet therapeutic strategies are limited. Development of novel medical therapies is challenged, however, by the limited understanding of the relevant pathophysiologic molecular pathways in the pulmonary vasculature of patients with PVH. Blockade of urokinase plasminogen activator receptor (UPAR) function has been demonstrated to attenuate the development of pulmonary arterial hypertension in animal models. We have previously identified UPAR as a marker of normal pulmonary veins. Pulmonary veins undergo many pathologic changes in PVH, including intimal and medial thickening (‘arterialization’) and occasional luminal occlusion. We hypothesized UPAR expression levels would parallel the venous remodeling in aortic banded (AOB) animals with PVH, and that these patterns would be validated in lungs of patients with left heart dysfunction and pulmonary hypertension. Aortic banding was performed as previously described. To image the pulmonary veins of both WT and AOB rats, a mixture of agarose and fluorescent beads was injected retrograde through the left ventricle while the aorta was clamped. Human lung tissue samples were obtained at time of left-ventricular assist device placement or heart transplant (IRB:09/2010-093). All tissue was fixed in 4% formaldehyde and embedded in paraffin for sectioning. Immunofluorescence was performed using commercial antibodies to UPAR (Santa Cruz Biotechnology, Calif.), heavy chain cardiac myosin (HCCM) (Abcam, MA), and α-smooth muscle actin (Abcam, MA). PVs were reliably identified and distinguished from arteries by retrograde filling with fluorescent beads and, in larger vessels, expression of HCCM. In control animals, expression of UPAR was confined to the pulmonary veins and not arteries. In the lungs both AOB animals and humans suffering from PVH, UPAR expression was upregulated in pulmonary arteries, and associated with vascular lesions in both the pulmonary arteries and veins. The development of novel medical therapies for PVH depends upon a firm understanding of the relevant underlying pathology and signaling pathways in the pulmonary vasculature. UPAR has been previously demonstrated to play a role in the experimental development of pulmonary arterial hypertension. Using a venous back-filling technique with fluorescent tags, the pulmonary arterial and venous compartments in both control and AOB animals were identified. The expression pattern of UPAR was characterized in both control and AOB animals. UPAR was upregulated in the pulmonary arteries of AOB animals, and associated with vascular lesions in both the pulmonary arteries and veins. These observations were validated in human lung tissue samples of patients with PVH. UPAR activity represents a pathway of interest in PVH, and further investigation is warranted to evaluate its role as a therapeutic target.

1.54 Exposure to cigarette smoke causes dysfunction

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Chronic obstructive pulmonary disease (COPD) is frequently associated with RV dysfunction, a poor prognostic marker. However, in animal models of increased afterload alone, such as PA banding, the RV function is preserved. Also, animal models have suggested that alveolar destruction by itself is not sufficient to cause smoking-induced cor pulmonale. These observations suggest that increased afterload alone or alveolar destruction is not sufficient to induce RV dysfuction. The objective of this study was to evaluate the effect of CS exposure on ventricular function and PA pressures. Male ARR mice were exposed to room air (RA) or cigarette smoke (CS) for three to six, at six hours per day, four days per week, using a TE-10 mouse smoking machine and 3R4F reference cigarettes with a total suspended particle concentration of ~120 mg/m³. Transthoracic echocarhoids was performed using Visutech Sonics Vevo 2100 imaging system. Lung compliance was assessed by measuring Cst using flexVents system. After measurement of lung mechanics, the lungs were fixed and H and E staining performed on sections. Microparticles were quantified in the serum using Nanosight N5S500. Three weeks of CS exposure increased lung compliance without significant change in mean linear intercept (MLI). Echocardiography showed impaired LV diastolic function with no effect on LV or RV systolic function, pulmonary acceleration time (PAT, a surrogate for mean PA pressure) or LV or RV mass. In contrast, six weeks of CS exposure...
resulted in impaired RV systolic (TAPSE in mm- RA: 1.1 ± 0.1, CS: 0.8 ± 0.1, P < 0.05) and diastolic function (TV e’ in mm- RA: -25 ± 1, CS: -19 ± 2, P < 0.05) and diastolic function [TV e’ in mm- RA: -25 ± 1, CS: -19 ± 2, P < 0.05] in lung compliance, [RA: 29 ± 1 μm CS: 33 ± 1 μm, P < 0.05] and in lung compliance, [RA: 0.04 ± 0.002 ml/cm H O, CS: 0.05 ± 0.006 ml/cm H O, P < 0.05] without changes in PAT or RV mass. Also, six weeks of CS exposure was associated with an increase in circulating microparticles (in 10^7/ml- RA: 5.8, CS: 10.3, P< 0.05). Exposure to CS causes abnormal RV function without elevated PA pressure. We speculate that these effects may be due to exposure to circulating CS constituents or microparticles released in response to CS.

1.55 Blockade of hypoxia-induced CA2+ release by acetazolamide (ACZ) in pulmonary arterial smooth muscle cells

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We have shown that ACZ, a carbonic anhydrase (CA) inhibitor which blunts hypoxic pulmonary vasoconstriction (HPV) in isolated lungs and live animals, dose-dependently inhibits the increase in PASMC intracellular calcium concentration ([Ca2+]i) induced by acute reductions in oxygen tension, by a mechanism unrelated to CA inhibition. Several studies now indicate that Ca2+ release from internal stores, with subsequent Ca2+ entry through store-operated Ca2+ channels (SOCC), is important for generating the hypoxia-induced increase in PASMC [Ca2+]i. In this study, we tested whether the ability of ACZ to inhibit hypoxia-induced increases in [Ca2+]i, is related to an effect on SOCC or Ca2+ release. Using fluorescent microscopy and transiently cultured rat PASMCs loaded with the Ca2+-sensitive dye, Fura-2 AM, SOCC was activated by depletion of stores with cyclopiazonic acid in the presence of nifedipine and 0 mM extracellular Ca2+. While re-addition of Ca2+ (Ca2+ restoration) ACZ (100 μM) had no effect on SOCC activity. The effect of hypoxia (4% O2) on [Ca2+]i was measured in the absence of extracellular Ca2+ to isolate changes in [Ca2+]i, due to Ca2+ release. Under these conditions, hypoxia induced a rapid, transient increase in [Ca2+]i. In the presence of 100 μM ACZ, no increase in [Ca2+]i was observed in response to hypoxia. To test whether ACZ directly inhibits release from ryamodine receptors (RyR) or inositol triphosphate receptors (IP,R), we tested ACZ on Ca2+ release due to caffeine (RyR) and phenylephrine (IP,R). ACZ had no effect on either response. These results indicate that ACZ inhibits hypoxia-induced increases in [Ca2+]i, and thus HPV by blocking release from internal stores, but that the mechanism does not appear to include direct blockade of either RyR or IP,R.

1.56 Haplotype association mapping in 33 inbred mouse strains identifies genetic regions contributing to chronic hypoxia-induced pulmonary hypertension

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While mutations primarily in BMPR2 [with a relatively small number in a few other genes] have been identified in patients with both the heritable and idiopathic forms of pulmonary arterial hypertension (PAH), data suggest that additional genetic factors contribute to the etiology of the disease. Short of the necessary large patient cohorts to power genome wide studies that could lead to the identification of additional genetic factors, researchers are left with few alternatives for genetic studies of PAH. In an effort to circumvent the paucity of PAH patient cohorts available, we are performing genome wide studies in readily available strains of inbred mice. Mice from 33 different strains were housed under hypoxic conditions (10% O2) for four weeks. Equivalent numbers of age and sex-matched mice were maintained under normoxic conditions for all 33 strains. After the hypoxic exposure, all mice underwent right heart catheterization to measure right ventricular systolic pressure (RVSP). Hearts were dissected at necropsy to assess the degree of right ventricular hypertrophy (RVH) as determined by the ratio of the weight of the right ventricle (RV) as compared to the weight of the left ventricle and septum (LV+S). Hematocrit was measured via retro-orbital puncture. Due to the small size of some mice, RVSP could only be determined for 31 strains. Haplotype association mapping (HAM) was performed using efficient mixed-model analysis (EMMA) and a set of 132,000 SNPs in all strains to identify regions in the genome associated with any of the quantitative traits measured. The HAM included separate analyses for hypoxic and normoxic mice, as well as a “response to hypoxia” analysis taking into account the difference in RVSP (or RV/LV+S or hematocrit) after hypoxia as compared to normoxic controls. Associations reaching genome wide significance (P = 1 x 10−8) were identified for both RVSP and RV/LV+S. The most significant association for RVSP was identified on chromosome 8 (P = 2.34 x 10−10) while that for RV/LV+S was on chromosome 6 (P = 9.37 x 10−10). Additional significant associations were identified on other chromosomes. These data suggest that genetic factors contributing to RVH and increases in RVSP can be identified using genome wide methods in inbred mice. Ongoing efforts are underway to identify the specific loci involved with the goal of translating these findings to patients with PAH. Identification of additional genetic factors contributing to PAH can ideally reveal novel pathways involved in disease pathogenesis that will lead to the development of novel therapeutics for patient treatment.

1.57 Therapeutic potential of histone deacetylation inhibitors in pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is characterized by pulmonary vascular remodeling, a result of excessive cell proliferation and resistance to cell death. Epigenetic programming, dynamically regulated by histone acetylation, is an important mechanism for controlling cell proliferation and survival. Little is known about the contribution histone deacetylases (HDAC) activity to the changes in cell phenotype that occur during vascular remodeling. We demonstrate for the first time changes in the expression of HDAC proteins, specifically increased HDAC1 (Class I) and HDAC5 (Class II), in human IPAH lung. These data were replicated in lungs and RV from rats with hypoxia-induced pulmonary hypertension. The lack of change in HDAC expression in the kidneys from these animals links the observed changes in HDAC expression to the pathology/vascular remodeling of pulmonary hypertension rather than the hypoxic stimulus per se. Immunohistochemical assessment of human IPAH and chronic hypoxic rat lungs confirmed increased nuclear expression of HDAC1 and cytoplasmic expression of HDAC5 in the remodeled vessels, along with the proliferative marker Ki67. Chronic administration of the HDAC class I inhibitor, VPA, prevented hypoxia-induced pulmonary hypertension and attenuated the phenotype in the rat when administered after pulmonary hypertension had become established. A similar result was obtained with the broad spectrum HDAC inhibitor, SAHA. Both, VPA and SAHA inhibited PDCG-stimulated human smooth muscle cell proliferation and the hyper-proliferation of epigenetically altered bovine R- cells and fibroblasts in culture. The precise molecular mechanisms by which HDAC inhibition exerts its effects in these models remain to be elucidated, but changes in Bcl-2 and p21 expression in vivo and p21, FOXO3, eIF2, PDGFb, S100A4 and survivin In vitro support strongly a direct effect on cell division and survival. Downregulation of pro-inflammatory factors such as MCP-1, IL-6, SDF-1 may also be involved. Our results suggest that increased HDAC activity contributes to the vascular pathology of pulmonary hypertension. HDAC inhibitors, VPA and SAHA, may have therapeutic potential in PAH.
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1.58 Sustained endothelial injury to maintain hyperproliferation and apoptosis-resistance in pulmonary arterial hypertension

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The angi-obliterative process in the lungs of patients with pulmonary arterial hypertension (PAH) is characterized by over-expression of markers of proliferation and apoptosis resistance in the cells in the wall of small pulmonary arteries. A similar quasi-malignant phenotype of cells in the pulmonary vasculature has been demonstrated in the lungs of rats exposed to the VEGF-R blocker SU5416 and hypoxia (SuHx). In vitro experiments on pulmonary microvascular endothelial cells have suggested that cellular injury favors selection of hyperproliferative and apoptosis-resistant cell clones. We have recently shown high rates of cell death in SuHx lungs, long after the establishment of severe angio-oblation and pulmonary hypertension. In addition, reversal of angio-obliteration after treatment with the copper chelator tetrathiomolybdate was not only associated with less presence of hyper-proliferative and apoptosis resistant cells, but also with evidence for reduced rates of apoptotic cell death. Simultaneous high rates of cellular death and proliferation may be explained by the strong stimuli for cell proliferation provided by the presence of apoptotic cells (i.e., apoptosis-induced compensatory proliferation). We will propose a new theoretical concept of vascular remodeling in PAH, where the quasi-malignant switch of pulmonary vascular cells in PAH is not self-sustaining and requires persistent vessel wall cell activation and apoptosis. This hypothesis could link etiological concepts of vasoconstriction, flow, inflammation and apoptosis-resistance in the pulmonary vasculature and would even imply that anti-proliferative and/or anti-angiogenic drugs may contribute to further cell activation and death in PAH and may even worsen vascular remodeling. Experimental strategies to address this hypothesis will be discussed.

1.59 Determining treatment efficacy in pulmonary arterial hypertension

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The rationale for using vasodilator treatment in pulmonary arterial hypertension (PAH) is the premise that, via a reduction in pulmonary vascular resistance (PVR), unloading of the right ventricle (RV) will improve right heart function, exercise capacity and survival. We recently showed, however, that absolute changes in PVR after vasodilator therapy do not correlate with changes in RV ejection fraction (RVEF) and that a decrease in RVEF is associated with a poor outcome, irrespective of changes in PVR. This finding may be explained by the fact that treatment-associated decreases in PVR are frequently due to an increase in cardiac output (CO) rather than a decrease in mean pulmonary artery pressure (mPAP). As such, treatment may increase RV power output (mPAPxCO) rather than lower the demands on the RV. Treatment may thereby improve exercise performance while having undetermined implications for RV function and patient survival. Because of the nonlinear relationship between PVR and arteriolar diameter, a high PVR at baseline may allow a larger absolute change in PVR and CO with vasodilator therapy, thereby assigning larger treatment effects to patients with a poor prognosis and RV function. We analyzed hemodynamic data from the major clinical trials with PAH-specific therapy and showed that regardless of drug used, treatment resulted in a larger PVR decrease and CO increase after three months and one year in patients with a high PVR at baseline. We retrospectively confirmed the proportional relationship between baseline PVR and changes in PVR and CO in idiopathic PAH patients treated in our center (n = 80). Neither absolute nor relative changes in PVR and CO over one year of treatment were predictive of changes in RVEF or subsequent survival. We conclude that higher PVR at baseline predicts a greater subsequent decrease in PVR, irrespective of treatment. In addition, neither absolute nor relative changes in PVR and CO are suitable to compare and monitor the efficacy of vasodilator treatment in clinical studies and individual PAH patients.

1.60 Defective eNOS phosphorylation in idiopathic pulmonary arterial hypertension

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Idiopathic Pulmonary arterial hypertension (PAH) is a fatal disease characterized by impaired regulation of cardiopulmonary hemodynamics. Previous studies from our laboratory have shown that patients have reduced nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS). We hypothesized that the low NO production by IPAH is due to reduced activity of endothelial nitric oxide synthase (eNOS) in pulmonary arterial endothelial cell (PAEC). NO production in control PAEC (n = 6) is doubled upon bradykinin stimulation (BK, 1 µM) [nitrite pmol/mg/min, unstimulated: 0.14 ± 0.01; stimulated: 0.26 ± 0.01, P < 0.0001] whereas NO production changed only minimally in PAH PAECs (n = 6) [pmol/mg/min, nonstimulated: 0.15 ± 0.01, stimulated: 0.18 ± 0.01, P = 0.01]. Dimerization of eNOS which regulates dimerization of the inactive enzyme, was similar among control and PAH PAECs. Phosphorylation at different sites dictates eNOS activity; phosphorylation of serine 1177 (S1177) is required for eNOS activity, whereas threonine 495 (T495) phosphorylation inhibits eNOS activity. BK stimulation caused S1177 phosphorylation in PAH PAEC, but less than control cells (pS1177/total eNOS, control: 1.25 ± 0.01, PAH: 0.92 ± 0.03, P = 0.01). BK induced rapid dephosphorylation of T495 in control PAEC, but not in PAH PAEC (pT495/total eNOS, control: 0.11 ± 0.01, PAH: 2.1 ± 0.06, P = 0.001). These findings point to derangements in signal transduction events that control eNOS phosphorylation that causes loss of eNOS activity in PAH.

1.61 CD39/CD73-mediated immune responses in pulmonary arterial hypertension

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Severe pulmonary arterial hypertension (PAH) is a fatal, multifactorial vascular disease that is characterized by lung vascular remodeling, high pulmonary blood pressure and right ventricular hypertrophy. Currently there are no effective therapies. PAH is known to be associated with T cell dysfunction. It has been recently shown that regulatory T cells can limit vascular cell injury and prevent PAH, yet no studies are known so far in respect to the impact of the enzymes CD39 and CD73. Extracellular ATP (eATP) plays an important role in regulating vascular and immune responses. Levels of eATP are regulated by two plasma membrane ecto-nucleotidases: CD39 (apprase/NTPDase 1 that hydrolyzes ATP to ADP and AMP) and ecto-5’-nucleotidase CD73 (that hydrolyzes eAMP to adenosine (Ado)). While eATP is known to exert pro-inflammatory effects, extracellular Ado (eAdo) has been implicated in anti-inflammatory responses. Here we investigated levels and functional activity of CD39 and CD73 in the circulation, spleen and lungs from severe PAH rats in response to hypoxia and the contribution of CD39 expressing inflammatory cells to vascular remodeling. SD rats were exposed to hypobaric hypoxia (18,000 ft = 5,000 m altitude) for three weeks. A second group of animals was additionally injected with the VEGF receptor inhibitor SUGEN5416 (20 mg/kg BW dispersed in 0.5% CMC and 1% Tween 20) at Day 0 and Day 7 of hypobaric hypoxia. Pulmonary arterial pressures and cardiac output were measured by inserting a pulmonary arterial catheter. Hematocrit and ratio of right versus left ventricle plus septum (RV/(LV+S)) were determined. Expression levels of CD39 and CD73 were determined by real time PCR using ITD primers, immunohistochemistry and Western blot analysis. Additionally, the expression of CD73 has been determined in...
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1.62 Estrogens induce right ventricle-pulmonary vasculature uncoupling in female rat model of accelerated angioproliferative pulmonary hypertension

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Pulmonary arterial hypertension (PAH) is debilitating disease that more frequently occurs in women. Yet, women have relatively preserved right ventricle (RV) function and survival compared to men with PAH. The effects of sex hormones on RV function in PAH are unknown. Therefore, we examined the effects of gender, estrogen deficiency and exogenous estradiol on RV hyper-trophy and PAH in our novel female rat model of accelerated PAH with plexiform (PLX) lesions. Adult male (M) and intact female rats (F), ovariec-tomized female rats (OVX), and OVX rats receiving estradiol via osmotic minipump (OVX+E2) were given VEGF antagonist SU 5416 (200 mg/kg s.c.) and exposed to hypoxia (10% O2) for 21 days. On Day 21, animals were examined for RV peak systolic pressure (RVPSP) and RV and left ventricle – pulmonary vasculature uncoupling: While estrogens adversely affect endothelial vascular remodeling and exacerbate PAH they attenuate RV remodeling. The female rat model of accelerated angioproliferative PAH therefore more closely mimics the pathogenesis of disease in humans. Further investigation of the role of estrogens and other sex hormones on RV function and survival in this model are warranted.

1.63 Hypoxia-induced mitogenic factor (HIMF/FIZZ1/RELMα)-induced pulmonary endothelial cell activation is critical for the later development of pulmonary hypertension and right heart dysfunction

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Hypoxia-induced mitogenic factor (HIMF), also known as found in inflammatory zone 1 (FIZZ1) and resistin-like molecule α (RELMα), belongs to a novel class of cysteine-rich secreted proteins. It exhibits mitogenic and chemotactic properties during pulmonary hypertension (PH)-associated vascular remodeling, as well as inflammatory and fibrogenic properties in the lung. We reported previously that HIMF/FIZZ1/RELMα promotes pro-inflammatory responses in the lung and lung vasculature, and that the Th2 cytokine interleukin-4 (IL-4) plays a crucial part of this phenomenon. Here, we investigated the mechanism of HIMF-induced pro-inflammatory responses, specifically in pulmonary endothelial cell (EC) activation and later development of PH. We injected recombinant (r) HIMF protein via tail vein into wild type (WT) and IL-4 KO mice, and we analyzed the expression of vascular inflammation- and PH-related gene expression in the lungs of each genotype. We also stimulated mouse primary pulmonary microvascular EC (PMVEC) with rHIMF In vitro, and examined the degree of EC death and the expression of vascular adhesion molecules. Lastly, we examined the effect of single rHIMF protein injection on the later development of PH and right heart dysfunction in vivo. Our results show that HIMF/FIZZ1/RELMα induced PH-related vascular inflammatory marker gene expression in a manner dependent on the Th2 cytokine IL-4. HIMF/FIZZ1/RELMα caused EC death at higher concentration and stimulated P-selectin expression in PMVEC. In addition, single systemic injection of rHIMF caused development of PH and showed significant decrease of mitochondria-related gene expression in the right heart. Our results suggest that HIMF/FIZZ1/RELMα plays a critical role in pulmonary inflammation by inducing EC activation and injury in the vascular beds. These phenomena may contribute to an increased risk for the development of PH, right heart failure, and other pulmonary vascular diseases.

1.64 Speckle tracking echocardiograph as a screening method for pulmonary hypertension in severe COPD

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Pulmonary hypertension (PH) is a common complication of chronic obstructive pulmonary disease (COPD). PH is associated with increased health care utilization and increased mortality in this patient population. Current echocardiographic measures used to estimate PAP are inadequate with poor predictive value for the diagnosis of PH. Speckle tracking echocardiography (STE) used to quantify myocardial deformation correlates well with right ventricular (RV) function and PAP as well as mortality in subjects with pulmonary arterial hypertension (PAH). We hypothesized that STE measurements would be more accessible than right ventricular systolic pressure (RVSP) values obtained from tricuspid regurgitation (TR) velocity and that RV strain obtained from STE would correlate with invasive hemodynamic measurements indicative of PH such as pulmonary vascular resistance (PVR). We retrospectively evaluated global RV and RV free wall longitudinal strain using STE on standard, four chamber apical views from subjects with GOLD stage IV COPD who had undergone echocardiography within 48 h of pulmonary artery catheterization (PAC). Complete data were available on 54 subjects. TR was identified in only 17 (31%) while RV strain could be obtained from at least one apical view in 44 (81%). RV free wall strain correlated linearly with PVR (r=0.17, p=0.02) using a PVR cut-off value of 3 WU, the receiver operating characteristic curve showed that an RV free wall strain of -24% was 92% sensitive and 42% specific at identifying pulmonary hypertension. Furthermore, RV strain correlates with invasive measurements of pulmonary vascular dysfunction. STE may be a means of improving screening for PH in this patient population, assisting in selection of those who merit further invasive studies such as RHC.

1.65 The TLR4/MyD88 signaling pathway is required for complement-dependent platelet activation chronic hypoxia-induced pulmonary hypertension
Abstracts

1.67 Hemodynamic and genetic analysis in children with idiopathic/heritable and congenital heart disease associated pulmonary arterial hypertension

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Idiopathic (I) pulmonary arterial hypertension (PAH) is rare in childhood and can be heritable (HPAH) caused by defects in transforming growth factor (TGFβ) signalling genes. The genetic background of congenital heart defects associated with PAH (CHD-PAH) is less clear. The aim of this prospective study was to compare clinical and genetic findings in children with I/HPAH and CHD-PAH. Prospectively included were consecutive children with invasively confirmed diagnosis of I/HPAH or CHD-PAH. Assessment of family members, pedigree analysis and systematic screening for mutations in the genes bone morphogenetic protein receptor 2 (BMPR2), ACVRL1, endoglin, SMAD1, SMAD5, and SMAD9 were performed. We included 19 children with I/HPAH (6.3 ± 4.7 years) and 11 with CHD-PAH (7.2 ± 4.5 years). Two Mutations in BMPR2 and ACVRL1, respectively, and three not yet described unclassified sequence variants (ACVRL1 n = 1; SMAD9 n = 2) were found in I/HPAH children. One ACVRL1 mutation has not been described before. In CHD-PAH patients 1 BMPR2 mutation and two unclassified sequence variants (endoglin n = 1, BMPR2 n = 1) were found. Carriers of genetic mutations and sequence variants with pathologic functional impact had a significantly lower PVR (926.96 ± 2520.53, P = 0.003) than patients with no mutation or silent sequence variants. Mutations and unclassified variants with functional impact in different TGFβ signalling genes occurred in 21% of I/HPAH patients and 37.5% of patients with CHD-PAH and may influence the clinical status of the disease. Therefore, genetic analysis in children with various forms of PAH is important, may be of clinical and prognostic relevance, and shows the complexity of the genetic background. The role of the newly identified sequence variants has to be further analyzed.

1.68 β2 adrenergic receptor polymorphism and gene expression are associated with risk of development of and disease severity in scleroderma associated pulmonary arterial hypertension

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Little is known about the role of neurohormonal dysfunction in the pathophysiology of pulmonary arterial hypertension (PAH), particularly with respect to PAH-related to scleroderma (SSc-PAH). Single nucleotide polymorphisms (SNPs) in the β2-adrenergic receptor (ADRB2) gene are associated with cardiovascular disease and specifically, risk of development of left heart failure (LHF). Similarly, expression of related genes in peripheral blood mononuclear cells (PBMC) has been associated with disease severity in LHF. Therefore, we sought to 1) determine whether previously validated SNPs in ADBR2 were associated with the risk of development of PAH in SSc, and 2) characterize the gene expression of ADBR2 in treatment-naïve SSc-PAH patients. Three hundred fifty-five
SSc patients without PAH and 103 SSc-PAH patients provided blood for genetic analyses. Several ADBR2 SNPs (n = 13) with previous functional significance in cardiovascular disease, were examined for their association with the risk of development of PAH in SSc at the single locus level using PLINK. Fifteen of the SSc-PAH patients, who were treatment-naïve at enrollment, also provided PBMCs for gene expression analyses using Illumina high-density BeadArrays. Pearson correlations between ADBR2 and clinical variables were calculated along with P values and false discovery rates (FDR). There was a significant association between the ADBR2 SNP Arg166Gly in the promoter region (rs17779257) and development of PAH in SSc (P = 0.03). Gene expression profiles showed a strong positive correlation with ADBR2 expression and cardiac output (r = 0.81, P < 0.0001, FDR <0.01). Preliminary results in this cohort suggest that functional SNPs in ADBR2 may be associated with the risk of development of PAH in SSc and that gene expression of ADBR2 is associated with disease severity. Given the known associations between ADBR2 and LHF, further study is warranted.

1.69 Pulmonary arterial hypertension induces gene expression changes in the right ventricle in advance of right ventricular failure that are more severe in female rats

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Right ventricular (RV) failure is the leading cause of morbidity and mortality in patients with pulmonary arterial hypertension (PAH), and females represent up to 75% of patients with PAH. However, most animal models of PAH focus on male rats precluding an analysis of sex-specific changes in RV adaptation or dysfunction. We analyzed genome-wide mRNA expression patterns in the RV of both female and male rat models of severe PAH to determine whether changes occur prior to the onset of RV failure, whether these changes resemble those characteristic of left ventricular (LV) failure, and whether there are sex-specific biological differences in RV failure. Six-week-old female and male rats underwent left pneumonectomy or sham surgery followed by 50 mg/kg MCT seven days later to induce severe, neointimal PAH. Rats underwent transthoracic echocardiography and continuous ambulatory invasive right heart hemodynamic monitoring. Cardiac tissue was harvested and RNA expression profiles were generated by microarrays from female (n = 3) and male (n = 4) rats with PAH 10 days following MCT, and from female (n = 4) and male (n = 4) control rats. Experimental rats exhibited significantly elevated pulmonary pressures but grossly normal RV size and function prior to sacrifice. One hundred ninety-five genes were differentially expressed in the RV of rats with PAH relative to normal control rats. These genes were involved in calcium signaling, myocyte contraction, mitochondrial function, extracellular matrix remodeling, cell proliferation, and cell membrane and cytoskeleton structure. Expression changes in Emp3, Fnl, Hspb1, Mgp, S100a4 and Timp1 were confirmed by real-time quantitative PCR in RV. Expression of these genes was unchanged in the LV. In general, female PAH rats exhibited more extreme gene expression changes than male PAH rats. We have documented gene expression changes in RV of rats with PAH prior to the appearance of significant RV enlargement. These changes resemble those occurring in LV failure but appear to be more severe in female relative to male rats.

1.70 Integration of genome-wide microRNA and mRNA expression profiles in pulmonary arterial hypertension and pulmonary hypertension associated with idiopathic pulmonary fibrosis

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PAH is a life-threatening condition characterized by pulmonary arteriolar remodeling, progressive elevation of pulmonary artery pressure, and right heart failure. The genomic mechanisms of PAH remain ill defined. We have recently reported genome-wide lung tissue mRNA expression changes associated with PAH and pulmonary hypertension (PH) associated with idiopathic pulmonary fibrosis (IPF). We hypothesize that microRNAs regulate many of these mRNA expression changes. We therefore determined genome-wide microRNA expression changes. Using microarrays, we generated microRNA expression profiles in lung tissue from subjects with PAH (n = 18), PH associated with IPF (n = 5), and normal controls (n = 10). Expression of 23 microRNAs was significantly increased in PAH relative to normal controls, with miR-101*, miR-33a and miR-144 and miR-410 appearing to be key players in the pathogenesis of PAH. mRNA targets were predicted for 21 of these 23 microRNAs using two independent computational models and Ingenuity Pathway Analysis and these data were integrated with our previously published mRNA expression data from the same subjects. Our analysis suggested that microRNAs were involved in the pathogenesis of PAH by altering cellular growth and proliferation, protein ubiquitination, and TGF-β (BMPR2, BMP1A, ACVR1, SMAD2/4), ERK/MAPK, β-adrenergic, and PDGF signaling. This is the first study to integrate genome-wide microRNA and microRNA expression data in PAH. The present study identifies a set of microRNAs that appear to regulate many of the changes in mRNA expression and perturbation of biological pathways that we previously observed in PAH. We also successfully validated the application of a novel computational model for mRNA target prediction on clinically acquired mRNA and microRNA expression data.

1.71 WNT-signaling pathway in right ventricular remodeling

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Right ventricular (RV) remodeling comprises multiple adaptation mechanisms to increased pressure- or volume-overload, which in concert determine RV performance, as well as clinical outcome in patients with pulmonary hypertension. It is believed that in the pressure-overloaded hearts a re-expression of neonatal genes, e.g., WNT signaling molecules is triggered. In our study, we employed two animal models of RV remodeling: Monocrotaline (MCT) model of pulmonary hypertension and pulmonary artery banding (PAB) model. To characterize global expression changes that occurred in our animal model RVs, microarray experiments were performed. Comparative, microarray based transcriptome analysis of RV remodeling identified upregulation of Frizzled receptors in the remodeled RVs, suggesting the involvement of WNT canonical pathway. In accordance, we found significant upregulation of several WNT signaling molecules including β-catenin, GSK3β, and Frizzled receptors in the RVs, as well as in the primary cardiac fibroblasts (CFs) isolated from PAB and MCT rats. Further addressing the influence of WNT signaling on CFs, we found that stimulation of CFs with WNT3a resulted with increased collagen expression and β-catenin knockdown caused decreased collagen synthesis. Similarly, WNT3a induced CFs proliferation and β-catenin knockdown caused decreased proliferation rates of these cells. Importantly, pulmonary artery banding of BAF-Gal mice containing a beta-catenin-activated LacZ reporter, resulted in increased β-catenin activation in remodeled RVs, as compared to sham operated animals. We can conclude that WNT signaling is important...
for the development of RV remodeling and may eventually offer innovative treatment strategies.

1.72 Sex and hemodynamics in pulmonary arterial hypertension

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Female sex is the best established risk factor for pulmonary arterial hypertension (PAH), yet women have better survival compared to men with PAH. This so-called "estrogen paradox" is as yet unexplained. We sought to determine if sex was associated with differences in baseline hemodynamics in patients with PAH. Methods: We conducted a pooled analysis from 11 randomized placebo-controlled trials submitted to the US Food and Drug Administration for approval. We excluded one trial which included background PAH therapy (PHIRST-1) and individuals with missing data. Results: The final study sample included 1,933 participants. After adjustment for age, race, height, weight, diagnosis, and study, the mean right atrial pressure (mRAP) was 1.5 mmHg (95% CI 0.8 – 2.2 mmHg, P < 0.001) higher; the mean pulmonary artery pressure (mPAP) was 2.5 mmHg (95% CI 0.7 – 4.4 mmHg, P = 0.01) higher, and the pulmonary vascular resistance (PVR) was 0.8 Wood units (95% CI 0.8 – 1.7 Wood units, P = 0.06) higher for men compared to women. There were no significant differences in cardiac output (CO), cardiac index (CI), or mean pulmonary capillary wedge pressure (PCWP) between sexes. Conclusions: Well-characterized males with untreated PAH had higher mRAP, mPAP, and possibly PVR at baseline compared to females. These hemodynamic differences may account for some of the disparate outcomes between men and women with PAH. Sex variable male female P value number of participants 420 1,513 age, year 50.0 ± 15.1 48.0 ± 14.8 0.01 race/ethnicity, n (%) Caucasian 365 (86.9) 1,201 (79.4) 0.01 African-American 13 (3.1) 79 (5.2) Asian 7 (1.7) 46 (3.0) Hispanic 33 (7.9) 171 (11.3) Other 2 (0.5) 16 (1.1). Height, cm 173.9 ± 8.1 160.4 ± 7.3 < 0.001 Weight, kg 80.5 ± 16.3 69.8 ± 17.6 < 0.001 diagnosis, n (%) idiopathic/primary 303 (72.1) 908 (60.0) <0.001 connective tissue disease 61 (14.5) 428 (28.3). Congenital heart disease 30 (9.0) 143 (9.5). Anorexigen use 1 (0.2) 12 (0.8). HIV Infection 6 (1.4) 4 (0.3). Other 11 (2.6) 18 (1.2). Baseline hemodynamics† mRAP, mmHg 9.5 ± 5.8 8.6 ± 5.4 0.001 mPAP, mmHg 54.1 ± 15.5 53.4 ± 15.4 0.64 CO, L/min 4.5 ± 1.7 4.2 ± 1.4 <0.001 CI, L/min/m2 3.1 ± 2.8 2.8 ± 1.5 0.80 mPCWP, mmHg 9.3 ± 3.5 9.0 ± 3.5 0.22 PVR, Wood units 11.5 ± 6.1 12.5 ± 7.0 0.02 data are shown as mean ± standard deviation or percentage. †Unadjusted values.

1.73 Distribution of radial distensibility in canine pulmonary vasculature

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The progression of pulmonary arterial hypertension (PAH) is typically assessed by the mean pulmonary artery blood pressure (mPAP) and pulmonary vascular resistance, but loss of conduit pulmonary artery compliance is a better predictor of mortality (Gan 2007). Loss of distensibility throughout the vasculature may also contribute to disease progression. In human studies, a global distensibility is typically measured from pressure-flow curves (Reeves 2005, Blyth 2005, Argiento 2010). Here we sought to establish a method for quantifying local changes in pulmonary vascular distensibility that could be used clinically. Two metrics of local distensibility were computed from magnetic resonance imaging (MRI) and digital subtraction angiography (DSA) of the pulmonary vasculature of six healthy female beagles under anesthesia; all procedures were IACUC approved. Both metrics are based on the change in diameter with a change in pressure, which was created by injecting polymer microbeads into the right ventricle. Then, \( \beta = (\Delta \text{pressure} – \Delta \text{diameter})/\Delta \text{pressure} \) and \( \alpha = \beta/\Delta \text{diameter} \) were calculated for six segments along the left and right principal pathways where \( \Delta \text{pressure} \) is the change in mPAP from pre to post. Best-fit diameters and distance from the main pulmonary artery were calculated from 3-D renderings of MRI (not shown) and DSA data in Mimics. As expected, \( \beta \) decreased with distance for both the right and left principal pathways. The decrease in \( \beta \) with distance was nearly linear for the right (R² = 0.938) but more hyperbolic for the left (R² = 0.679), which suggests a difference in both structure and function. \( \alpha \) was nearly constant with distance for both principal pathways, with an average value of 1.40 ± 1.02 %/mmHg, which is close to the 2%/mmHg found previously for healthy humans (Reeves 2005; Argiento 2010). These local measurements of distensibility agree well with previous measurements of global distensibility. While here we altered mPAP by embolization, the method could be used clinically using MRI in conjunction with any maneuver that acutely alters mPAP, such as exercise. By quantifying the distribution of distensibility in human lungs in healthy and diseased states, more insight may be gained into the mechanics and mechanisms of PAH progression.