353. Pulmonary circulation: basic mechanisms, animal models and cell biology

P3163
Hypoxia-induced pulmonary hypertension: Synergistic effects of sildenafil and erythropoietin in mice
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The term pulmonary arterial hypertension (PAH) describes a group of diseases characterized by elevated pulmonary arterial pressure. The cause can be due to several vascular changes, including pulmonary remodeling. If left untreated, patients might die from right heart failure within an average of three years. The present study was designed to investigate single and combination therapy with erythropoietin (epo) and sildenafil on hypoxia-induced PAH.
Mice were randomized, first in a normoxic and a hypoxic group and second to receive saline, epo, sildenafil or epo and sildenafil. Epo was injected three times per weekly (500 IU/kg) and sildenafil daily (10 mg/kg). The animals were exposed to three weeks of either hypoxia (10% oxygen) or normoxia, after which they underwent the different treatments for an additional two weeks. Immunohistochemistry was performed to elucidate changes in morphology. Plasma levels of cardiotrophin-1 and atrial natriuretic peptide (ANP) were measured. The pulmonary pressure was estimated using right heart catherization.
On average the hypoxic mice lost approximately 20% of their body weight. This was reduced to 5% for the group receiving the combination treatment. The hypoxia-induced increase in right ventricular hypertrophy and medial wall thickness of pulmonary arterioles was significantly attenuated with the combination therapy. Similar results were also observed for cardiotrophin-1 and ANP levels.
The combination treatment with epo and sildenafil demonstrated an improvement in the clinical outcome in hypoxia-induced PAH in rodents, superior to that observed for either drug given alone.

P3164
Myocardial prostacyclin effects in experimental acute afterload-induced right ventricular failure
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It has been suggested that prostacyclin improves patients with pulmonary hypertension through direct myocardial effects. In an experimental model of acute right ventricular (RV) failure on increased afterload, we previously reported that prostacyclin did not present with significant inotropic effects, but that there might have been a trend as assessed from improved RV-arterial coupling (Kerbaul et al. AJRCCM 2007).
Therefore, we further explored the pathobiology of acute RV failure with/without prostacyclin infusion.
Dogs were randomized to a sham-(n=8) or to a 90-min pulmonary artery constriction (PAC)-operation (n=15). In 7 dogs with persistent RV failure, prostacyclin was infused 30-min after banding release. After euthanasia, RV tissue was sampled for pathobiological evaluation.
90-min PAC increased RV gene expressions of interleukin (IL)1β, monocyte chemoattractant protein (MCP)1, IL6, VCAM1, and decreased expression of IL10. No changes in RV gene expressions of IL1α, macrophage inflammatory protein (MIP)1α and ICAM1 were observed after PAC. Protein expressions of IL1β and IL6 were increased in the failing RV, while IL10 protein expression was decreased. Pro-inflammatory IL6/IL10 and pro-apoptotic Bax/Bcl2 ratios were increased in the failing RV. Increased diffuse macrophage recruitment was observed within the failing RV. Prostacyclin decreased RV gene expressions of IL1β, MCP1, MIP1α, VCAM1 and increased IL10 expression. Prostacyclin decreased RV gene IL6/IL10 and Bax/Bcl2 ratios, and IL1β protein expression compared to PAC group.
Acute load-induced persistent RV failure appears to be related to an activation of inflammatory processes which seems to be limited by prostacyclin.

P3165

Screening for biomarkers in pulmonary hypertension

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Introduction: Pulmonary hypertension (PH) is a progressive and fatal disease. The gold standard for diagnosing PH and estimating prognosis is the invasive method of right heart catheterization. To date no biomarker is available to prove or exclude the diagnosis of PH.

Aims and objectives: The aim of this study is to identify and validate new biomarkers for PH.

Methods: Plasma from the pulmonary artery banding (PAB) and the monocrotaline (MCT) rat model, and corresponding sham and control animals (n=9), was used for 2D-gel electrophoresis (2D-GE) and MALDI-TOF-MS analysis. Further, plasma changes of interesting candidates were confirmed by ELISA. Human study population consists of patients with idiopathic pulmonary arterial hypertension (n=40), PH associated with collagen vascular disease (n=45), pulmonary venous hypertension (n=44), chronic thromboembolic PH (n=45), and non-PH controls (n=34).

Results: The spot density analysis of 2D-GE and identification by MALDI-TOF-MS revealed 7 proteins significantly changed in PAB vs. sham, and 15 proteins in MCT vs. control group. Complement component 4 (C4) and complement inhibitory factor H (CFH) were upregulated in PAB and MCT. ApoE was changed 15-fold in MCT plasma, but not in PAB. The analysis of the human samples revealed no significant difference in mean plasma ApoE between the patient groups (119.4±10.3, 147.6±11.6, 116.8±9.9, 110.2±8.3 μg/ml) and controls (135.3±14 μg/ml).

Conclusions: Despite published data on the role of ApoE in PH and the significant changes in rats, ApoE seems not suitable as biomarker for PH in humans. Other candidates identified by mass spectrometry will be evaluated for their potential as biomarker.

P3166

Effects of soluble guanylate cyclase (sGC) stimulation in guinea pigs chronically exposed to cigarette smoke

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Background: The nitric oxide-cyclic GMP signaling pathway is altered in pulmonary vessels of patients with chronic obstructive pulmonary disease (COPD). Activation of sGC improves vascular remodeling in experimental models of pulmonary hypertension. The effects of sGC stimulation in COPD remain unsettled. The aim of the study was to evaluate the low dose effects of the sGC stimulator BAY 41-2272 in a guinea pig model of COPD.

Methods: 24 guinea pigs were exposed to cigarette smoke (CS) (6 cigarettes/day, 5 days/week) during 3 months. Half of them received BAY 41-2272, 3mg/kg daily (CS+BAY group), and the other half received vehicle (CS group). 16 guinea pigs, sham exposed to CS, were treated with BAY 41-2272 (Sham+BAY group) or vehicle (Sham group), and served as controls. Pulmonary artery pressure (PAP), cardiac output, % of muscularized intrapulmonary arteries (<50μm), airway resistance (enhanced pause, Penh), and mean interseptal distance (Lm) were assessed at the end of the study.

Results: Results are shown in the table:

	CS	CS+BAY	Sham	Sham+BAY
PAP (mmHg)	8.3±1.3*#	7.6±2.1*#	5.5±0.7	6.0±1.3
Cardiac Output (mL/min)	82±35#	88±28#	101±23	124±32
Muscularized vessels (%)	71±10*#†	54±13*#	28±12	31±8
Penh (AUC)	4.1±1.4*	4.1±1.4*	2.9±0.2	3.3±0.5*
Lm (μm)	75.3±5.5*#†	62.3±8.3	57.6±4.1	59.5±5.6

*p<0.05 vs Sham, #p<0.05 vs Sham+BAY, †p<0.05 vs CS+BAY.

Conclusions: In the guinea pig, stimulation of sGC prevents the development of pulmonary vascular remodeling and emphysema induced by CS. At the given low dose, these effects were not translated into a reduction of pulmonary hypertension or airflow obstruction.

Supported by grant FIS IS09-00536; Bayer Pharma AG kindly donated BAY 41-2272.

P3167

Contribution of stem-like cells to angioproliferative pulmonary arterial hypertension in the SU5416/chronic hypoxia model

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Severe pulmonary arterial hypertension (PAH) is a disease with high mortality and no curative treatment. The goal of our study was to investigate the potential contribution of stem-like cells to the angioobliteration in the SU5416/chronic hypoxia (SuHx) model of severe PAH.

Animals were treated according to the SuHx protocol and underwent sequential labeling with two halogenated thymidine analogues to investigate asymmetrical cell division, and treatment with the CXCR4 inhibitor AMD3100 (day 1-21) or recombinant human G-CSF (day 14-21).

After three weeks, we identified by immunofluorescence/confocal microscopy in the angioproliferative lesions of the SuHx rat model of severe PAH cells expressing markers of pluripotent stem cells and multipotent differentiation. We also showed that lesion cell division in SuHx animals was asymmetrical. The CXCR4 inhibitor AMD3100 reduced pulmonary artery muscularization and obliteration of medium size vessels in the animal model without greatly impacting PAH. This was associated with a reduction in the numbers of c-kit+ cells in the vessel.

We further show that G-CSF-induced cell mobilization from the bone marrow did not affect the PAH, but caused enhanced pulmonary artery muscularization together with pulmonary neovascularization instead, associated with an augmented accumulation of c-kit+ cells in and around the vessels.

In conclusion, our data indicate that putative stem-like cells, which express pluri- and multipotency markers, contribute to the pulmonary angioproliferation. Successful treatment of severe angioobliterative PAH will likely require a better knowledge of the role of such cells in the pathobiology of PAH.

P3168

Effect of long-term imatinib treatment in a severe preclinical PAH rat model: An insight into pulmonary perfusion using a novel casting method

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Introduction: Pulmonary arterial hypertension (PAH) is characterized by increased vascular tone and remodeling of pulmonary vasculature. Imatinib- a potent inhibitor of tyrosine kinases, reverses pulmonary vascular remodeling in animal models of PAH and improves haemodynamics in selected PAH patients. However, the efficacy of long-term Imatinib administration on pulmonary arteriopathy and impaired vascular perfusion observed in severe PAH patients is not known.

Aims: To examine the effects of long-term Imatinib (100mg/kg, PO QD) treatment on hemodynamics, and vascular perfusion in a severe preclinical rat model of PAH.

Methods: Wistar Kyoto rats were treated once with SU5416 (20mg/kg, s.c.) and exposed to hypoxic conditions for 2 weeks (+ controls), + 8 weeks normoxia, + 4 weeks imatinib treatment (100mg/kg, q.d.). Haemodynamic parameters were analysed to determine extent of PAH pathology. Perfusion mapping was performed via lung vascular corrosion casts.

Results: Rats exposed to chronic hypoxia/SU5416 developed severe right ventricular pressure (RVP >94mmHg) with decline in cardiac output (CO; -10.18 ml/min) compared to normoxic controls.Treatment with imatinib reduced all measures of PAH pathology (RVP ≤71 mmHg, CO; 6.42 ml/min). Macroscopic analysis of lung corrosion casts showed a large rarefaction of terminal branches of pulmonary vasculature (vascular pruning). Imatinib treatment greatly attenuated this decline.

Conclusion: The above data further supports the beneficial effects of imatinib on PAH pathology. We show for the first time that Imatinib restores pulmonary vascular perfusion in a clinically relevant model of advanced PAH.

P3169

Cell cooperation between human fibrocytes and endothelial progenitor cells during neoangiogenesis is driven by the CXCR4 pathway

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Idiopathic pulmonary fibrosis (IPF) is associated with a vascular remodeling process. Fibrocytes are a distinct population of blood-borne cells that coexpress hematopoietic cell antigens and fibroblast products, which have been shown to contribute to organ fibrosis. The purpose of this study was to test the hypothesis

that fibrocytes may cooperate with endothelial progenitors to induce angiogenesis.
We successfully isolated fibrocytes from blood of IPF patients. The characterization of fibrocytes used flow cytometry, real time q-PCR and confocal analysis. We investigated the interaction between fibrocytes and cord blood derived endothelial colony forming cells (ECFC) angiogenic potential in vitro and in vivo in a preclinical model of vascularization.
We show for the first time the formation of functional microvascular beds in immunodeficient mice when human ECFC and fibrocytes isolated from IPF patients were co-implanted in matrigel plugs. Evaluation of implants after 2 weeks revealed an extensive network of blood vessels containing erythrocytes. Secreted media from fibrocytes enhances SDF-1/CXCR4 pathway in ECFC in vitro. Blocking CXCR4 in vivo in implants significantly inhibited blood vessel formation. Finally, we confirmed the relevance of these data by showing that vessels close to fibrotic areas in biopsy specimens from IPF patients expressed high levels of CXCR4, in contrast to control lungs.
Circulating fibrocytes might be involved in vascular remodeling process observed in patients with fibrotic disease and should represent a useful biomarker for fibrosis progression.

P3170
miR 16 modulates human pulmonary artery smooth muscle cell phenotype
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Introduction: Pulmonary artery hypertension (PAH) is progressive fatal disease characterized by excessive proliferation of human pulmonary artery smooth muscle cells (hPASMCs) and obliteration of small pulmonary arteries, leading to severe pulmonary hypertension and right ventricular failure.
Objective: Serotonin mediates hPASMCs proliferation through serotonin transporter (SERT). Recently micro RNAs have been shown to be involved in pathogenesis of PAH. miR16 targets SERT and Cyclin D1.Our previous results show that miR-16 was down regulated in hypoxic PAH mice. We hypothesize that over expression of miR16 can alter PAH phenotype. In the present study, we investigated the phenotypic consequences of overexpression of miR16 in hPASMCs in vitro.
Methods: hPASMCs were commercially obtained and cultured according to manufacturer instructions. Cells were transfected with plasmid containing miR16 expressing or control vector by electroporation. Post transfection, cells were treated with different concentrations of serotonin and qPCR, immunoblot was performed to assess the effects of miR-16 on PAH phenotypic markers.
Results: hPASMCs transfected with miR16 decreased the expression levels of cyclin D1, SERT when compared to controls. In addition, proliferation markers such as proliferating nuclear cell antigen (PCNA), calponin a phenotypic marker was significantly suppressed in miR16 over expressed hPASMCs.
Conclusions: miR-16 over expression down regulates SERT and CyclinD1 levels. Most importantly miR-16 modulates hPASMCs phenotype which is a major hallmark in PAH, implicating a potential therapeutic role in PAH.
This work was supported by RO1HL105932NIH, AHA09SDG2260957.

P3171
Macrophage migration inhibitory factor (*MIF*) promoter polymorphisms are associated with favorable hemodynamic indices in systemic sclerosis-associated pulmonary arterial hypertension
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Rationale: Inflammatory mediators are increasingly associated with pathogenesis in pulmonary arterial hypertension (PAH). We have previously observed a 3.7-fold increase in serum levels of the pro-inflammatory macrophage migration inhibitory factor (MIF) in PAH patients. *MIF* promoter polymorphisms (-173*C, -794CATT$^{5-8}$) have been associated with disease susceptibility or phenotype in several inflammatory syndromes. We hypothesized that *MIF* promoter polymorphisms may influence PAH development or severity.
Methods: Genomic DNA was isolated from 117 European-American PAH patients, including idiopathic (IPAH; N = 35) and systemic sclerosis-associated PAH (SSc-PAH; N = 82), healthy European-American controls (N=264), and SSc patients without PAH (N=343). Allele and genotype frequencies for the *MIF* -173*C single nucleotide polymorphism and the -794CATT$^{5-8}$ variable nucleotide tandem repeat were compared between PAH patients and controls, and were compared with initial hemodynamic indices and survival in PAH patients.
Results: We found no significant difference in the frequencies of either *MIF* promoter polymorphism between controls and PAH patients. SSc-PAH patients with the *MIF* -173*C polymorphism had higher cardiac output (P=0.04), cardiac index (P=0.003), and stroke volume index (P=0.01) at diagnosis. Neither polymorphism predicted survival in PAH patients.
Conclusion: The *MIF* -173*C polymorphism may improve initial hemodynamics in SSc-PAH. However, *MIF* promoter polymorphisms do not predict PAH susceptibility or survival. These results suggest that *MIF* may function as a disease-modifying gene in SSc-PAH.

P3172
Dexamethasone induces anti-remodelling effects in rat pulmonary arterial smooth muscle cells
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Inflammation is increasingly recognised in pulmonary arterial hypertension (PAH). Given that dexamethasone (dex) reverses PAH and pulmonary vascular remodelling in established rat monocrotaline (MCT)-induced PAH, we tested contributing mechanisms in pulmonary arterial smooth muscle cells (PASMC). Nuclear localisation of the p65 subunit of NF-κB was used as a marker of inflammation in MCT-exposed rats.
Methods: PASMC from control and MCT 6-week old male Wistar rats were treated with increasing dex concentrations. Proliferation (3H-thymidine) and apoptosis (Hoechst nuclear staining; DNA fragmentation ELISA) assays were performed (n=5 per group). Immunohistochemistry for caspase 3 and p65 was performed on paraffin-embedded lungs from day 28 MCT-alone, MCT+dex-treated and control rats.
Results: Dex reduced proliferation of PASMC at all concentrations with a maximal effect seen at 10-7M (3H-thymidine counts/minute 84997±6802 to 1993±3135, $p<0.0001$). Dex increased serum starvation-induced PASMC apoptosis as determined by Hoechst staining and DNA fragmentation in a time- and concentration-dependent manner, reaching a plateau after 72hrs with 10-7M dex (0.40±0.17 to 1.29±0.54, $p<0.001$). In rat lung, caspase immunostaining was increased in the PASMC layer in dex-treated MCT rats vs. MCT-alone controls (0 (0-40)% vs. 58.3 (1-100)%, $p<0.0001$). Finally, nuclear p65 was reduced in PASMC and endothelial cells in MCT-treated rats at all dex doses studied.
Conclusion: Dexamethasone reduces proliferation and augments apoptosis in rat PASMC in vitro, and reduces activation of NF-κB within vascular cells. These actions, at least in part, may explain the reversal of PAH by dex seen in the rat MCT-PAH model.

P3173
Exogenous BMPR2 modulates TGF-β signalling in human small airway epithelium and smooth muscle cells
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Introduction: Idiopathic Pulmonary Arterial Hypertension (IPAH) is a fatal disease characterized by reduced BMPR2 and increased TGF-β expression.
Aims and objectives: To investigate the effects of upregulation of BMPR2 on TGF-β signalling in human small airway epithelial cells (hSAEC) and pulmonary artery smooth muscle cells (hPASMC)
Methods: The BMPR2 gene was delivered to both hSAEC and hPASMC using a plasmid construct or Adenovirus vector, then TGF-β signalling events assessed.
Results: In hSAEC, TGF-β upregulated P-38MAPK phosphorylation and vimentin but downregulated pancytokeratin, consistent with epithelial to mesenchymal transition (EMT). In preliminary studies, prior transduction to upregulate BMPR2, ameliorated the TGF-β-induced increase in vimentin. In hPASMC, TGF-β did not affect P-38MAPK, but led to a substantial increase in phosphorylated ERK 42/44 and Smad2, and reduced phosphorylation of Smad1/5/8. These effects were largely prevented by BMPR2 transduction.
Conclusions: To date, these findings suggest that BMPR2 gene delivery reduces TGF-β-induced epithelial to mesenchymal transition and TGF-β signalling in smooth muscle cells.
Supported by: Australian NHMRC.

P3174
Increased right ventricular cardiomyocyte stiffness in patients with pulmonary arterial hypertension
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Introduction: This study aims to determine whether changes in right ventricular

(RV) cardiomyocyte contractile apparatus are involved in RV failure secondary to pulmonary arterial hypertension (PAH).
Methods: Maximal force and passive stiffness were determined in membrane-permeablized RV cardiomyocytes isolated from RV tissue obtained after heart-lung transplantation of PAH-patients and non-failing donors. Maximal force was measured at maximal Ca2+-activation, while cardiomyocyte passive stiffness in relaxing solution (low Ca2+ concentration).
– The role of β-Adrenergic receptor signaling on cardiomyocyte passive stiffness was mimicked by determining passive stiffness after PKA incubation.
Results:
– No significant differences were found in cardiomyocyte maximal force in PAH patients and donors.
– Passive stiffness was significantly increased at all sarcomere lengths in PAH patients compared to donors.
– PKA incubation partially restored RV cardiomyocytes passive tension in PAH patients to donor values.

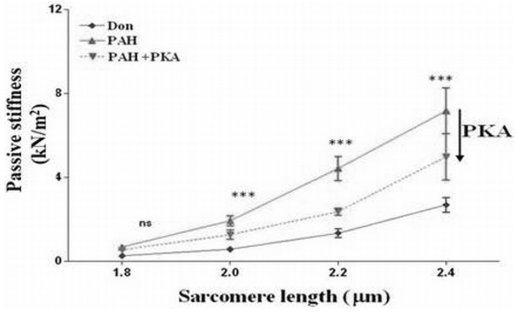

Figure 1. PKA effect on PAH RV cardiomyocites.

Conclusions: Our study demonstrates increased RV cardiomyocytes passive stiffness in PAH patients, partially restored by PKA incubation. This finding suggests that reduced β-adrenergic receptor signaling plays an important role in the development of RV diastolic stiffness in PAH patients.

P3175
BMPR2 gene delivery shifts intracellular Smad activation profile
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PAH is a fatal disease associated with decreased BMPR2 and increased TGF-β mediated cellular signalling. We have shown that BMPR2 upregulation in pulmonary endothelium reduces PAH in vivo, but the downstream signalling effects are unknown. BMPR2 is thought to signal primarily via Smad 1/5/8 and TGF-β via Smad 2/3, thus we hypothesised that BMPR2 upregulation may alter the relative activity of these pathways.
Aims and objectives: To assess the effect of BMPR2 upregulation on downstream Smad signalling in endothelial cells, and the response to BMP ligands.
Methods: Human Microvascular Endothelial Cells (HMVEC) were transduced with an Adenoviral vector carrying the BMPR2 gene, and then stimulated with BMP7 or 9.
Results: Efficient upregulation of BMPR2 was confirmed by western blot and persisted beyond 48 hours. Incubation with BMP7 and 9 showed similar increases in p-Smad1/5/8 and Smad 5 at 24 hours following transduction, but by 48 hours after transduction, BMP7 effects were significantly greater than BMP9. Interestingly, BMP9 stimulated cells had increased Endoglin compared to BMP7 stimulated cells at 48hrs. Both ligands lead to reduced p-Smad 3 with a similar profile.
Conclusion: BMPR2 upregulation shifts the intracellular signalling profile toward Smad 1/5/8, and away from Smad 3 consistent with an alteration in downstream TGF-β/BMPR2 pathways, which may in part explain the effects on PAH seen in vivo. There may be differential effects depending on the BMP ligand involved, which requires further analysis.
Supported by NHMRC & NHF.

P3176

WITHDRAWN

P3177
The role of bromodomain-containing protein 4 in the constitutive activation of nuclear factor-kappa B in endothelial cells from patients with pulmonary arterial hypertension
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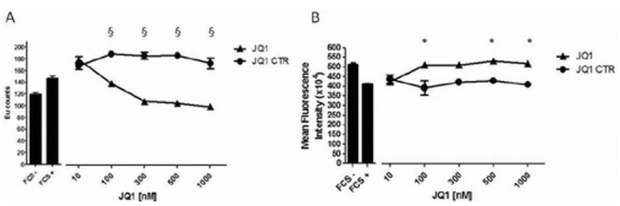
Background: Pulmonary arterial hypertension (PAH) is characterized by a progressive increase in pulmonary vascular resistance leading to right heart failure and death. Pulmonary endothelial cells (P-ECs) are well known as producers of cytokines and chemokines essential in the recruitment of inflammatory cells to the lungs, and a constitutive activation of the nuclear factor-kappa B (NF-κB) signaling pathway in P-ECs has been recently described in PAH. RelA lysine-310 acetylation of NF-κB generates a specific docking sites for bromodomain-containing protein 4 (Brd4). We hypothesize that Brd4 through an NF-κB-dependent mechanism contributes to the hyperproliferative and proinflammatory phenotype in P-ECs in patients with PAH.
Aim: The aim of the study was to evaluate the in vitro effect of Brd4 inhibition using the selective inhibitor JQ1 on proliferation and apoptosis in P-ECs.
Methods and results: The effect of JQ1 on P-ECs proliferation was established by assessing the incorporation of BrdU. We found a strong anti-proliferative effect of JQ1 in P-ECs (Fig. 1A). We also demonstrated that JQ1 induces caspase-3 activity in P-ECs resulting in increased apoptosis (Fig. 1B).



Conclusion: Selective Brd4 inhibitors, such as JQ1, may represent novel therapeutic agents for the treatment of PAH. Further work is necessary to explore this hypothesis.

P3178
The combined therapy of HGF and G-CSF for pulmonary hypertension in rats
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Introduction: Despite advances in therapy of PAH, the treatment and prognosis remain poor.
Objectives: This study was to investigate whether expression of HGF through the transplantation of genetically modified MSCs combined with G-CSF could offer therapeutic benefit.
Methods: Three weeks after monocrotaline, SD rats were randomly divided into PAH (n=10), MSCs (transplantation of 5×10^6 MSC transfected with an empty adenovirus vector,n=10), HGF (transplantation of 5×10^6 MSCs transfected with Ad-HGF,n=10), G-CSF group (100μg/kg daily for 5 days,n=10),and HGF+G-CSF group (transplantation of 5×10^6 MSCs transfected with Ad-HGF and G-CSF, 100μg/kg daily for 5 days,n=10). Three weeks later, hemodynamic, histomorphology, endothelial and angiogenesis function were detected, involving serum levels of TGF-β, ET-1 and protein level of VCAM-1and MMP-9.
Results: Compared with HGF, G-CSF and PAH group, HGF and HGF+G-CSF group have significantly less right ventricular hypertrophy, PASP and mPAP ($p<0.05$). Histologically, vascular smooth muscle cell proliferation and extra cellular matrix were also significantly decreased ($p<0.05$). The vascular density of

HGF+G-CSF group is higher than control group ($P<0.05$). The TGF-β and ET-1 concentration in the plasma of pulmonary hypertension rats showed markedly decreased in HGF group and HGF+G-CSF group ($P<0.05$). Furthermore, HGF induced gene and protein expression of VCAM-1 and HGF treatment together with G-CSF synergistically stimulated MMP-9 expression.
Conclusion: Transplanted HGF-MSCs combined with G-CSF could offer synergistic therapeutic benefit for the treatment of pulmonary hypertension. This study illustrate mechanisms underlying the synergistic effect of G-CSF and HGF combination.

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A critical role for p130^{Cas} in the progression of pulmonary hypertension in humans and rodents
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Pulmonary arterial hypertension (PAH) is a progressive and fatal disease characterized by pulmonary arterial muscularization due to excessive pulmonary vascular cell proliferation and migration, a phenotype dependent upon growth factors and activation of receptor tyrosine kinases (RTKs). p130^{Cas} is an adaptor protein involved in several cellular signaling pathways that control cell migration, proliferation and survival.We hypothesized that in experimental and idiopathic PAH p130^{Cas} signaling is over-activated, thereby facilitating the intracellular transmission of signal induced by fibroblast growth factor (FGF)2, epidermal growth factor (EGF), and platelet derived growth factor (PDGF). In iPAH patients, levels of p130^{Cas} protein are higher in the serum, in walls of distal pulmonary arteries, in cultured smooth muscle (PA-SMCs) and pulmonary endothelial cells (P-ECs) than controls. These abnormalities in the p130^{Cas} signaling were also found to be in the chronically hypoxic mice and monocrotaline-injected rats. We next obtained evidence for convergence and amplification of the growth-stimulating effect of EGF, FGF2 and PDGF signaling pathways via p130^{Cas} signaling pathway. Finally, we found that daily treatment with each of the EGF-R inhibitor gefitinib, the FGF-R inhibitor dovitinib and the PDGF-R inhibitor imatinib started 2 weeks after a subcutaneous monocrotaline injection substantially attenuate the abnormal increase in p130^{cas} and ERK1/2 activation and regress established PH. Our findings demonstrate that p130^{Cas} signaling plays a critical role in iPAH by modulating pulmonary vascular cell migration, proliferation and by acting as an amplifier of RTKs downstream signals.