oversee vascular homeostasis, are increased in IPAH. We hypothesized that IPAH CD133+ cells are derived from a predominantly myeloid bone marrow (BM) stem cell that promote the pathologic pulmonary vascular remodeling. CD133+ cells sorted from IPAH, unaffected family members or healthy control BM aspirates, were engrafted into immunodeficient mice and analyses were performed after 7 weeks or if mice became morbid. Recipients of PAH or unaffected family member CD133+ cells had higher engraftment and myeloid differentiation as indicated by higher human CD45+ and CD33+ cells. Mice engrafted with PAH CD133+ cells had higher plasma von Willebrand Factor (vWF) as compared to control mice, indicating endothelial/platelet activation, and demonstrated higher morbidity and mortality. Histological examination of organs revealed cardiac and pulmonary remodeling in PAH recipients, but no remodeling in the control group. Tissue remodeling included in situ thrombi, muscularization of pulmonary arterioles, right ventricular hypertrophy, infarction and ischemia. PAH recipients had a 2-fold increased microvessel density in lungs but not in heart or liver. Lung microvessel density correlated to circulating human progenitors in the mouse circulation. Collectively, the data suggest that the development of PAH precedes CD133+ proangiogenic myeloid progenitor cell proliferation that contributes to the progression and/or pathogenesis of PAH, possibly through endothelial cell injury/platelet activation.

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Prolonged overcirculation-induced pulmonary arterial hypertension, as a cause of right ventricular failure

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Three-month chronic systemic-to-pulmonary shunting in piglets has been reported as an early pulmonary arterial hypertension (PAH) model with preserved right ventricular (RV) function. We hypothesized that prolonged shunting would induce severe PAH and RV failure. Fourteen piglets were randomized to a sham operation or the anastomosis of the left innominate artery to pulmonary arterial trunk. Six months later, animals underwent hemodynamic evaluation followed by tissue sampling for pathobiological assessment. Prolonged shunting resulted in increased pulmonary vascular resistance and pulmonary arteriolar medial thickness, while cardiac output was decreased. The ratio of end-systolic to arterial elastances (Ees/Ea) decreased from 1.4±0.1 to 0.7±0.1, suggesting a RV failure. At RV tissue level, pro-apoptotic Bax/Bcl2 ratio and caspase-3 activation were upregulated, with an inverse correlation between RV Ees/Ea and pro-apoptotic Bax/Bcl-2 ratio.



In the failing RV, IL-1a, IL-1β and TNF-a expressions were locally increased, along with increased circulating levels of TNF-a. RV capillary densities were similar between the 2 groups, while gene expressions of VEGF and angiopoietin-2 were decreased in the failing RV tissue. Prolonged left-to-right shunting in piglets does not further aggravate PAH, but is a cause of RV failure, which appears related to an activation of apoptosis, hypertrophy and inflammation.

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Expression and role of the nerve growth factor NGF in pulmonary hypertension

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Introduction: Our previous studies showed an increased NGF expression in animal models of pulmonary hypertension (PH) and suggested a role for NGF in various aspects of this disease. We have studied here expression of NGF and its receptors in human PH, and investigated whether administrating anti-NGF blocking antibodies in vivo prevented some pathological aspects in a rat PH model.

Methods: NGF and inflammatory cytokine secretion was assessed by ELISA in human pulmonary arteries (PA) from controls or from patients with secondary PH. Expression of NGF receptors (TrkA and p75NTR) was assessed by Western blotting. In the rat, PH was induced by monocrotaline (MCT, J1, 60mg/kg ip), with or without administration of anti-NGF blocking antibodies (J0, J2, J7, 10µg/kg ip). Pulmonary arterial pressure (Pap) and Fulton index were assessed at J28. PA medial wall thickness was evaluated on lung sections after hematoxylin and eosin staining. PA reactivity to phenylephrine or prostaglandin F2 α (PGF2 α) was assessed ex vivo.

Results: Significant increase in expression of NGF and its receptors was observed in PA from patients with secondary PH compared to controls. In the MCT-treated

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Late-breaking abstract: Intracellular mechanisms behind the effect of C-reactive protein on proximal vascular cells of CTEPH patients Marijke Wynants¹, Rozenn Quarck¹, Bart Meyns², Marion Delcroix¹

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Chronic thromboembolic pulmonary hypertension (CTEPH) is associated with vascular remodeling and inflammation. Our latest results showed that C-reactive protein (CRP) could contribute to vascular remodeling and endothelial dysfunction in pulmonary vascular cells of CTEPH patients.

We aimed to investigate the intracellular mechanism responsible for the effect of CRP on CTEPH pulmonary vascular cells.

Pulmonary proximal arterial endothelial (EC) and smooth muscle cells (SMC) were isolated from patients with CTEPH. After stimulation with CRP, total RNA was extracted from CTEPH-EC and CTEPH-SMC and first stand cDNA was generated. A RT² profiler PCR Array (SABioscience) was used to evaluate the expression of 84 key genes related to NFkB-mediated signal transduction.

Different genes from the NFkB pathway were up- or downregulated in CRP-stimulated CTEPH-EC and CTEPH-SMC. In CRP-stimulated EC isolated from 5 different CTEPH patients, the serotonin receptor 1B was significantly downregulated (p=0.0089) compared to not stimulated CTEPH-EC. CRP significantly downregulated the toll-like receptor 4 (p=0.032) and inhibitor of kappa B light polypepetide (p=0.025) in CTEPH-EC. In CRP-stimulated SMC isolated from 4 different CTEPH patients, mucosa associated lymphoid tissue 1 (p=0.038) and B-cell lymphoma 10 (p=0.012) were significantly upregulated.

These results suggest an involvement of the NFkB pathway in mediating the effects of CRP on vascular cells of CTEPH patients.

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Human pulmonary arterial hypertension bone marrow-derived CD133+ myeloid progenitors induce vascular remodeling in immunodeficient mice Kewal Asosingh¹, Samar Farha¹, Alan Lichtin², Jacqueline Sharp¹, Deepa George¹, Brain Graham⁵, Micheala Aldred⁴, Karl Theil³, James Loyd⁶, Rubin Tuder⁵, Serpil Erzurum¹. ¹*Pathobiology, Cleveland Clinic, Cleveland,* United States

Severe remodeling of the pulmonary artery is the hallmark of idiopathic pulmonary artery hypertension (IPAH). Myeloid CD133+ proangiogenic progenitors, which rat, anti-NGF blocking antibodies significantly reduced Pap, Fulton index, cytokine secretion, PA medial wall thickness, and totally prevented PA hyperreactivity to phenylephrine or PGF2 α .

Conclusion: Our data show that expression of NGF and its receptors are increased in human PH. Moreover, administration of anti-NGF blocking antibodies in the rat in vivo partially prevented PH by modulating PA hyperreactivity, inflammation and remodelling. These results therefore confirm that targeting NGF may be of therapeutical interest in this disease.

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Inflammatory processes in load-induced right ventricular failure

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Background: Transient increase in pulmonary arterial (PA) pressure has been shown to induce a persistent right ventricular (RV) failure characterized by a RV-arterial decoupling, associated to activation of apoptotic pathways and local overexpression of TNF-alpha (Dewachter *et al.* Crit Care Med 2010; 38: 1405-13). **Objectives:** We hypothesized therefore that inflammatory cytokines might contribute to the development of persistent RV failure in this "pulmonary hypertension crisis" model.

Methods: Sixteen dogs were randomized to a 90-min PA constriction- or to a SHAM-operation, followed 30 minutes later by hemodynamic measurements including effective pulmonary arterial elastance (Ea) to estimate RV afterload and RV end-systolic elastance (Ees) to estimate RV contractility determined by the single beat method (Brimioulle *et al.* Am J Physiol 2003; 284: H1625-30), but also blood sampling. After sacrifice of the animals, RV free wall was sampled to assess, by RTQPCR and ELISA, respectively gene and protein expressions of interleukin (IL)-1 beta, -6 and -10.

Results: The transient increase in PA pressure persistently increased Ea, and decreased Ees, Ees/Ea and cardiac output, indicating RV failure with altered RV-arterial coupling. As compared to the SHAM group, 90-min PA constriction increased RV relative gene and protein expressions of IL-1 beta and IL-6, and decreased RV relative gene and protein expressions of IL-10, an anti-inflammatory cytokine. The pro-inflammatory IL-6/IL-10 ratio was increased in the RV and in the serum in the PA constriction- compared to the SHAM- group.

Conclusions: Acute afterload-induced persistent RV failure appears to be related to local and systemic activation of inflammation.

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Right lung ischemia induces development of contra-lateral pulmonary vasculopathy

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Chronic thromboembolic pulmonary hypertension is due to mechanical obstruction by unresolved clots and vasculopathy in the non obstructed vascular lung regions. We tested whether flow induced vascular lesions or endocrine factors released by the ischemic lung account for development of vasculopathy in non obstructed regions.

3 groups of 5 piglets were studied 5 weeks after right pulmonary artery (PA) ligation (PAL group), right pneumonectomy (RP group) or right PA dissection (Sham group). We measured pulmonary vascular resistance, pulmonary arterial vasoreactivity and morphometry, and quantified gene expression of factors involved in vascular smooth muscle cell proliferation IGF, PDGF, VEGF and endothelium-dependent vasoreactivity pathways ET-1, ETAr, ETBr and eNOS.

As compared to RP, PAL animals developed pulmonary vasculopathy in the left lung as assessed by increase in pulmonary vascular resistances (p=0.0006), medial hypertrophy of the distal PA (p<0.0001), decreases in both maximal relaxation to acetylcholine (p=0.013) and eNOS gene expression (p=0.041). Left lung IGF (p=0.034), PDGF (p=0.0066) and VEGF (0.0105) gene expressions increased in the PAL group when compared to both RP and Sham. ETAr and ETBr expression was downregulated in both RP (p=0.048 and p=0.039) and PAL (p=0.033 and p=0.028) groups.

Pulmonary vasculopathy is absent in the remaining lung 5 weeks after pneumonectomy and developed in the non obstructed pulmonary territories 5 weeks after right PA ligation suggesting that factors released by the ischemic lung induced vascular remodelling in the contra-lateral lung. This endocrine regulation might implicate release of factors involved in vascular smooth muscle cell proliferation.

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p53-dependent, replicative cell senescence suppresses chronic hypoxia-Induced pulmonary hypertension in mice

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Introduction: Excessive telomere shortening limits both cell proliferation and

tissue regenerative capacity by inducing senescence, a permanent growth arrest mediated by the p53 pathway. The purpose of this study was to evaluate the susceptibility of mice with defective telomerase (TERC^{-/-}) to develop hypoxia-associated pulmonary hypertension (PH).

Methods: TERC^{-/-}, p53^{+/-} TERC^{-/-}, and wild-type (WT) mice at 12 weeks of age were randomly divided into two groups, one of which was maintained in room air (normoxia) and the other exposed to normobaric hypoxia (10% oxygen, 90% nitrogen) for 3 weeks. We evaluated pulmonary hemodynamics, vascular remodeling, and the p53-mediated response in the lung.

remodeling, and the p53-mediated response in the lung. **Results:** The TERC^{-/-} mice were efficiently protected against chronic hypoxiainduced PH as compared to WT mice. Interestingly, TERC^{-/-} mice exhibited marked increase in p53 protein levels under normoxia and lacked all signs of tissue remodeling associated to vascular cell proliferation seen in WT mice upon hypoxia treatment. Remarkably, p53 heterozygous TERC^{-/-} mice were fully susceptible to hypoxia-induced PH and developed all cell proliferation-related histopathological manifestations.

Conclusions: Taken together, these results show that a telomere-mediated, p53dependent, senescence in vascular tissues confers a strong protective effect on chronic hypoxia-induced PH in mice.

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Right ventricular oxygen supply and diffusion in human and experimental pulmonary hypertension

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Introduction: In pulmonary hypertension (PH), right ventricular (RV) oxygen supply has to increase to meet the higher oxygen consumption due to the high RV power output. To investigate how the RV adapts to meet these requirements, we studied the oxygen supply and intracellular diffusion in terms of capillarization and myoglobin content in patients with PH. In addition, we compared healthy rats to a rat model with stable PH (preserved cardiac output) and progressive PH (RV failure) to answer the question whether adaptations in oxygen handling are characteristic for the failing RV or develop during progression of the disease.

Methods: RV tissue was collected at autopsy of 10 diseased PH patients and 10 control patients who died from a left ventricular myocardial infarction. In rats, stable PH and progressive PH were induced by monocrotaline 40 and 60 mg/kg, respectively. RV cardiomyocyte cell size, capillary density and myoglobin content were determined.

Results: RV cardiomyocyte cell size is strongly increased in PH patients compared to controls (PH: 824 ± 125 vs con: $353\pm97 \ \mu\text{m}^2$, p<0.001), while capillary density was decreased (PH: 557 ± 110 vs con: 1119 ± 195 capillaries/mm², p<0.001). RV myoglobin content was significantly reduced (PH: 0.56 ± 0.18 vs con: 1.0 ± 0.34 a.u., p<0.05) thus showing reduced intracellular oxygen diffusion. Similar results were found in the failing RV of progressive PH rats. Furthermore, stable PH rats showed an intermediate state from healthy to failing RV.

Conclusion: Oxygen supply to and diffusion within the cardiomyocytes is reduced in both failing human and rat RV. This is characteristic for the failing PH RV since stable PH rats maintained supply and diffusion.