475. Pulmonary circulation: basic mechanisms

4550
Pulmonary lymphoid neogenesis in idiopathic pulmonary arterial hypertension
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Background: Idiopathic pulmonary arterial hypertension (IPAH) patients present circulating autoreactive T cells against vascular wall components. Pathogenic antibodies may be generated in tertiary (i.e. ectopic) lymphoid tissues (tLTs).

Aims and objectives: To assess how frequent are tLTs in IPAH lungs as compared to controls and flow-induced PAH (Eisenmenger syndrome –E(S)–) and to identify local mechanisms responsible for their formation, perpetuation and function.

Methods: tLTs composition and structure were studied by multiple immunostainings. Cytokines/chemokines and growth factor expression was quantified by real-time PCR and localized by immunofluorescence. The systemic mark of pulmonary lymphoid neogenesis was investigated by flow cytometry analyses of circulating lymphocytes.

Results: As opposed to controls and E(S), IPAH lungs contained perivascular tLTs, compartmentalized in T cell areas with high endothelial venules and dendritic cells. Lymphocyte survival factors, such as IL-7 and PDGF-A, were expressed in tLTs as well as the lymphomagenetic cytokine/chemokines, lymphotactin-α/β, CCL19, CCL21 and CXCL13, which might explain depletion of circulating CCR6+ and CXCR5+ lymphocytes. The presence of germinal center centroblasts, follicular DCs, activation-induced cytidine deaminase and IL21+PD1+ T follicular helper cells in tLTs together with CD138+ plasma cells accumulation around remodeled vessels in areas of Ig deposition argued for local immunoglobulin (Ig) class switching and ongoing Ig production.

Conclusions: We highlight the main features of lymphoid neogenesis specifically in the lungs of IPAH patients with IPAH providing new evidence of immunological mechanisms in the evolution of this fatal condition.

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NMDA-type glutamate receptors contribute to the development of pulmonary hypertension
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Background: The NMDA receptor (NMDAR) is present in the three peripheral systems involved in Pulmonary Arterial Hypertension (PAH): immune, vascular systems and heart, but it’s unknown whether it plays a role in the pathophysiology of this disease.

Aims: 1) Highlight the presence of NMDARs and its agonist glutamate in the pulmonary vascular wall of PAH patients. 2) Search for deregulations of this signaling pathway in PAH. 3) Test the effect of NMDAR antagonists in a rat model of PAH.

Methods: NMDARs and glutamate were detected by flow cytometry and confocal microscopy. Effects of two NMDAR antagonists (memantine 100mg/kg/day from day 1 to 21 and MK-801 3mg/kg/day from day 14 to 21) were studied in a rat model of monocrotaline-induced PAH. We measured hemodynamic parameters, pulmonary vascular remodeling, right heart hypertrophy, levels of circulating markers of endothelial cell (EC) dysfunction (ICAM-1 and E-selectin) by ELISA and memantine by liquid chromatography and tandem mass spectrometry.

Results: 1) GluN1, the obligatory subunit of NMDARs, is expressed in the walls of pulmonary arteries in PAH patients, particularly in ECs that show enhanced proliferation to glutamate. 2) In human and experimental PAH, pulmonary arterial smooth muscle cells accumulate glutamate. 3) Chronic and curative administration of memantine and MK-801 respectively, improve all parameters of PAH in the experimental model, including a reduced EC dysfunction. 4) Improvement of PAH was due to the inhibition of GluN1/GluN2A and/or GluN1/GluN2B NMDARs.

Conclusion: Glutamatergic signaling occurs via NMDARs in the pathophysiology of PAH and may represent an innovative therapeutic target.

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4552
Effect of fasudil on the bleomycin-induced pulmonary fibrosis and hypertension in mice
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Background: Rhoa/Rho kinase (ROCK) is a pathway in regulating vascular tone and vascular remodelling in pulmonary hypertension (PH). It has been shown to be altered in the bleomycin-induced pulmonary fibrosis (PF) and PH in mice. However, the exact mechanism by which it leads to PF and PH remains to be clarified.

Objectives: The present study aimed to assess whether fasudil, a ROCK inhibitor, is able to inhibit PF and PH induced by bleomycin in mice.

Methods: Male C57Bl/6 mice were randomized into 3 groups: G1 (saline), G2 (bleomycin) and G3 (bleomycin + fasudil). Fasudil (3.3U/kg) was given intra-tracheally (day 0) and fasudil (30mg/kg) intraperitoneally from day 1 to day 21 during 21 days. Right ventricular systolic pressure (RVSP) was measured by RV puncture at 7, 14, and 21 days, followed by sacrifice and lung and heart sampling for collagen analysis.

Results: Pulmonary fibrosis was present at 7 days, and became more apparent at 14 days. RVSP increased at 14 days, accompanied by right ventricular hypertrophy. Fasudil improved survival, reversed PF and attenuated PH.

Conclusions: The efficacy of the ROCK inhibitor, Fasudil, suggests that Rhoa/ROCK is involved in causing PF and PH induced by bleomycin in mice.

4553
Polymorphisms in angiotensin converting enzyme gene are associated with risk of development of and disease severity in scleroderma-related pulmonary arterial hypertension
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Background: While 8-12% of patients with scleroderma (SSc) will develop pulmonary arterial hypertension (PAH), little is known about risk factors for this complication. Angiotensin converting enzyme (ACE) is associated with endothelial dysfunction and may play a role in susceptibility to vascular disease in SSc. We sought to identify polymorphisms in ACE gene that may contribute to risk of PAH in SSc.

Methods: A case-control study was performed in 916 patients of European descent. Of 458 SSc patients, 103 had right heart catheterization-proven PAH; the remainder did not have significant respiratory disease. Three single nucleotide polymorphisms (SNPs) in ACE gene (rs4293, rs3730025, rs4311) previously shown to be associated with cardiovascular disease, were examined. The relative frequency of SNPs and their relationship to presence of PAH and severity of PAH were assessed using Cochran-Armitage trend test with PLINK and linear regression for association between genotype and hemodynamics.

Results: A strong association was found between SNP rs3730025 and risk of PAH (P<0.009). Carriers of G allele of rs4293 had increased cardiac index (β=0.458, P=0.005) and decreased pulmonary vascular resistance (β=0.137, P=0.018).

Conclusion: In this SSc cohort, a coding SNP in ACE gene was strongly associated with presence of PAH. Further, presence of SNP rs4293 was associated with preserved cardiac function in SSc-PAH. Given the role of these SNPs in the function of ACE and the relationship between ACE and vascular function, further studies are warranted to investigate the role of these SNPs in the pathogenesis of PAH in SSc.

4554
Reduction in serotonin signaling via TPH inhibition attenuates the progression of PAH in mice with genetic ablation of endothelial BMPR-II
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Introduction: Genetic studies in familial PAH have revealed heterozygous germline mutations in the BMPR2 gene and dysfunction of serotonin (5-HT) signaling has been implicated in other forms of PAH. Here we investigate a genetic model of PAH, where BMPR2 deletion is restricted to endothelial cells.
4555
Hypoxia-induced miR-130a is a novel repressor of BMPR2 gene expression in experimental pulmonary hypertension
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Introduction: Vascular remodelling, a pathogenetic feature of pulmonary hyper-
tension (PH), is associated with decreased expression of the bone morphogenetic
protein receptor type II (BMPR2). We recently demonstrated that the inhibition of
BMPR2 and TGFBR2 were applied to confirm direct miRNA – target interactions.
Using SYBR-Green real-time PCR. Reporter gene assays comprising the 3'UTR of
miR-130a were found to be significantly upregulated (p<0.05). NB analysis showed that miRNA-139 was
downregulated significantly mycd, sm 22a and calponin. We conclude that slug
of slug two-fold (p<0.05) whereas no changes were observed in this gene after
treatment with IL-1β or INF-g. Fully differentiated SMC treated with TNFa,
dowregulatet significantly mycd, sm22a and calponin. We conclude that slug
expression might be associated with a SMC proliferative phenotype induced by
inflammation. This SMC phenotype switching might contribute to the development
and progression of vascular disorders.
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4557
miR-20a, miR-125a, -130a, -130b in the 3'UTR of
BMPR2f/f;ALK1-Cre mice exposed to chronic hypoxia under
VEGFR inhibition (SU5416) for 3 weeks.

Results: Using a miRNA target prediction program we identified phylogenetically
conserved binding sites of miR-21, miR-125a, -130a, -130b in the 3'UTR of
BMPR2. Under hypoxic conditions the RNA levels of miR-21, miR-125a, and
miR-130a were found to be significantly upregulated (p<0.05) when compared
with normoxic controls. Consistent with previous reports the miRNA expression of
BMPR2 was significantly reduced in hypoxic lungs (0.79±0.24 fold, p=0.039)
and, most importantly, showed a negative correlation with the expression of miR-
130a (R²=0.28, p=0.04). By performing reporter gene assays we confirmed that
miR-130a directly targets the 3'UTR of BMPR2. Along this line, we identified the
BMPR2-related receptor TGFBR2 as another novel target of miR-130a.

Conclusion: We identified miR-130a as a novel regulator of BMPR2 expression
indicating an important role of miR-130a in the development of hypoxia-induced
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