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Pulmonary lymphoid neogenesis in idiopathic pulmonary arterial hypertension

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Background: Idiopathic pulmonary arterial hypertension (IPAH) patients present circulating autoantibodies 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 -ES-) 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 immunofluoresence. The systemic mark of pulmonary lymphoid neogenesis was investigated by flow cytometry analyses of circulating lymphocytes.
Results: As opposed to controls and ES, IPAH lungs contained perivascular tLTs, comprising B and 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 lymphorganogenic cytokine/chemokines, lymphotoxin-α/-β, CCL19, CCL20, 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 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 is 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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Effect of fasudil on the bleomycin-induced pulmonary fibrosis and hypertension in mice

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Background: RhoA/Rho kinase (ROCK) pathway is important 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). Bleomycin (3.3U/kg) was given intratracheally (day 0) and fasudil (30mg/kg/d) intraperitoneally from day -1 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 samplings 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.

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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.

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Reduction in serotonin signaling via TPH1 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

(ECs)-BMPR2$^{f/f}$;ALK1-Cre strain, aiming to determine a relationship between pathologies mediated by 5-HT and BMPR2 signaling.
Methods: We investigated the effects of p-chlorophenylalanine (pCPA; 200mg/kg-inhibits 5-HT synthesis by blocking tryptophan hydroxylase (TPH)), on hemodynamics, vascular remodeling and plasma signatures in wild-type (WT) and BMPR2$^{f/f}$;ALK1-Cre mice (BMPR2-KO) exposed to chronic hypoxia under VEGFR inhibition (SU5416) for 3 weeks.
Results: Pathobiology development profile of BMPR2-KO mice at 2 and 5 months of age, demonstrated that the older age group tended to have a higher frequency of increased right ventricular pressure (RVP) and hypertrophy (RVH). 2 month old BMPR2-KO mice exposed to chronic hypoxia/SU5416 developed higher RVP and RVH compared with WT controls. Normoxic WT and BMPR2-KO mice did not display significant changes in the above measurements when compared between groups. Treatment with pCPA reduced all measures of PAH pathology observed in chronic hypoxic/SU5416 treated BMPR2-KO mice. These changes were observed in accordance with a fall in platelet rich plasma 5-HT.
Conclusion: Genetic ablation of the BMPR2 gene in pulmonary ECs is sufficient to predispose to PAH. Our data reveal interplay between BMPR2 signaling and the 5-HT system in ECs within PAH, leading to increased susceptibility to PAH progression.

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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 hypertension (PH), is associated with decreased expression of the bone morphogenetic protein receptor type II (BMPR2). We recently demonstrated that the inhibition of microRNA 20a (miR-20a) prevents vascular remodelling in hypoxia-induced PH. Here we assessed the role of miRNAs in the repression of BMPR2 gene expression in experimental PH.
Methods: The mouse model for hypoxia-induced PH was used. After 21 days of hypoxia, lungs were removed and assessed for miRNA and gene expression. RNA levels of miR-21, -25, -125a, -130a, -130b and BMPR2 were measured using SYBR-Green real-time PCR. Reporter gene assays comprising the 3'UTR of BMPR2 and TGFBR2 were applied to confirm direct miRNA – target interactions.
Results: Using a miRNA target prediction program we identified phylogenetically conserved binding sites of miR-21, -25, -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 to normoxic controls. Consistent with previous reports the mRNA 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 (R^2=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 negative regulator of BMPR2 expression indicating an important role of miR-130a in the development of hypoxia-induced PH.

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microRNAs are deregulated in pulmonary arteries from COPD patients
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Pulmonary vessel remodelling in chronic obstructive pulmonary disease (COPD) is associated with changes in smooth muscle cell (SMC) phenotype. MicroRNAs (miRNAs) regulate the expression of many genes controlling cell growth and differentiation. The aim of the study was to evaluate miRNAs expression in pulmonary arteries (PA) from COPD and control patients. We studied 29 PA from COPD (n=12), smokers (S) with normal lung function (n=10) and nonsmokers (NS) patients (n=7) who underwent lung resection. MiRNAs expression was assessed after RNA isolation by RT-PCR using taqman low-density arrays card A Set v3.0 (Applied Biosystems). MiRNAs expression was additionally evaluated by Northern Blot (NB) in primary human pulmonary artery endothelial (HPAE) and in human pulmonary artery smooth muscle cells (HPASMC) (Lonza) with both proliferative and contractile phenotype. MiRNAs were also studied in PA-derived SMC from patients. Expression of markers was assessed by Real Time PCR. The results showed a significant upregulation of miR-146 and miR-139, whereas miR-204, miR-149, miR-197, miR-487b and miR-485 were downregulated in PA from COPD respect to NS ($p<0.05$). NB analysis showed that miRNA-139 was specifically expressed in HPAE; miR204 in HPAE and in contractile SMC while the other miRNAs were abundantly expressed in SMC. Some miRNAs had differential expression levels in proliferative respect to a contractile SMC, suggesting a role of those miRNA in the phenotypic switch of SMC. We conclude that vascular remodeling in COPD might be linked to an alteration of SMC miRNAs expression. Supported by grants FIS 09/00536 and 10/02175, SEPAR-2009 and 2010. MMM is recipient of a Sara Borrell grant from ISC III, Spain.

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Slug can contribute to the phenotypic modulation of smooth muscle cells
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Recently, we have found that remodeled pulmonary arteries have increased expression of slug, a transcription factor related with transitional changes in cell phenotype. Smooth muscle cells (SMC) may show high plasticity switching from a contractile (fully differentiated) to a proliferative phenotype (dedifferentiated). The aim of the study was to investigate in human pulmonary SMC the expression of slug during the phenotypic switching in an in vitro model of cell differentiation, and also after exposure to inflammatory cytokines. We analyzed the expression of slug and specific markers of mature SMC in three cell culture states: 70% confluence (dedifferentiated),100% confluence (partially differentiated) and 4 days after reaching confluency (contractile/fully differentiated). In cytokine assays SMC were starved overnight and subsequently stimulated with TNFa, IL1-b and INF-g, 10ng/ml each. Slug expression decreased 30% and 50% ($p<0.001$) in partially and fully differentiated cells, respectively. Contractile SMC showed a significant upregulation ($p<0.05$) of the SMC markers myocardin (mycd), Sm22a and calponin. Treatment of contractile SMC with TNFa during 48h increased the expression of slug two-fold ($p<0.05$) whereas no changes were observed in this gene after treatment with IL1-b or INF-g. Fully differentiated SMC treated with TNFa, downregulated 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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