3033 Protective role of lysyl oxidases in aortic aneurysm progression in MFS

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Marfan syndrome (MFS) is an autosomal dominant disorder caused by point mutations in the fibrillin-1 (FBN1) gene that associates with high risk for the development of thoracic aorta aneurysms. Recent research on novel functions of FBN1 has shown dysregulation of transforming growth factor-β (TGF-β) as a central mechanism in the pathogenesis of the aortic pathology. Clinical trials based on the inhibition of TGF-β signaling are being currently evaluated as a potential therapeutic approach. However, little is known about the precise pathogenic TGF-β downstream targets that contribute to the disease progression. TGF-β has been shown to induce the expression of lysyl oxidases (LOX), a family of copper-dependent amino oxidases that play a critical role in the biogenesis of connective tissue matrices by cross-linking collagen and elastin, thereby providing the fibers with their physical properties of strength and elasticity.
In this work we explore the contribution of LOX family to the development of the aortic disease in MFS. Ascending aorta from MFS patients were analyzed for the expression of LOX isoforms. Aneurysmal aortic tissue from MFS showed enhanced expression of LOX and LOXL1, particularly in regions displaying collagen accumulation. In order to study the role of LOX in the progression of aortic disease, we administered β-aminopropionitrile (BAPN), a specific inhibitor of LOX activity, to wild type (WT), heterozygous $Fbn1^{C1039G/+}$ and mgR/mgR mice (MFS mice). LOX inhibition increased aortic growth in $Fbn1^{C1039G/+}$ and mgR/mgR mice, causing premature death in mgR/mgR mice. This finding correlates with enhanced activation of canonical (phospho-Smad2) and noncanonical (ERK1/2) TGF-β pathways.
This work defines a critical role for LOX in aneurysm progression in MFS, and highlights this family of matrix remodeling enzymes as a possible therapeutic target to slow down the progression of the aortic dilatation in MFS.

3034 Combined Radiation and Burn Injury Causes Pulmonary Fibrosis

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Radiation-induced pneumonitis and fibrosis has been observed in radioactive disaster survivors. Severe radiation injuries frequently occur in conjunction with burn wounds. Since both are known to independently cause pulmonary complications, we developed a model to study the effects of combined radiation injury (CRI) in which C57BL6 mice were divided into 5-5.5 Gray (Gy) total body irradiation, 15% total body surface area scald burn, radiation followed by burn (CRI), or sham controls. We previously reported that 48 hours post-injury, lungs from CRI mice showed excessive inflammation characterized by elevated leukocyte infiltration and heightened levels of neutrophil and monocyte chemokines, compared to either insult alone. This early pulmonary congestion and inflammation could potentiate and promote lung fibrosis. To test this hypothesis, we examined lungs from mice sacrificed 7 months post-injury. Histologically, we noted that radiation-exposed mice had alveolar collapse, increased cellularity, and interstitial thickening, which was markedly worsened by CRI. Trichrome staining showed increased collagen deposition in mice exposed to radiation, which was dramatically elevated after CRI. We measured newly synthesized collagen and found that CRI mice had 150% more than radiation alone, twice that of burn alone, and 3-fold more than controls ($p < 0.05$). Correspondingly, CRI mice had increased respiratory rate (f) as measured by plethysmography. Surprisingly, no differences were observed in levels of most pro-inflammatory, angiogenic and fibrogenic mediators tested by multiplex bead array, including IL-15, IL-18, LIF, M-CSF, MIG, MIP-2, PDGF2, and VEGF, with the exception of bFGF, which was 83% reduced in the lungs of CRI mice than control, and 73% lower than radiation alone ($p<0.05$). We plan to examine earlier time-points to elucidate the mechanisms by which combined injury exacerbates the fibrogenic response.

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3035 Inflammation and rapid-onset fibrosis in mouse lungs induced by multi-walled carbon nanotubes

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The production of multi-walled carbon nanotubes (MWCNT) has been significantly increased in recent decades due to their widespread applications, which has led to a concern regarding their impacts on human health because of the similarities of MWCNT to asbestos fibers. Recently, pulmonary fibrosis was associated with the major effects of MWCNT on health, but with limited progress in understanding the extent of MWCNT exposure in initiation and progression of lung fibrosis and in identifying the molecules that contribute to this pathologic consequence. Here, we showed MWCNT were a potent fibrotic agent that induced rapid-onset fibrosis upon single exposure to low doses 7 days post-exposure in C57BL/6 mice, indicated by histological analysis and increased expression of fibrosis marker genes, such as αSMA and Col1a1. The MWCNT exposure elicited significant inflammatory response evidenced by inflammatory infiltration in the lungs and markedly increased PMN count and lactate dehydrogenase activity in the BAL from fibrotic lungs. To understand the underlying mechanism, we determined the expression of several groups of molecules including the genes involved in inflammatory immune responses, extracellular matrix remodeling and cytotoxicity, by PCR array analysis. Elevated gene expression was verified in the lungs and in fibrotic foci, which occurred mainly in the regions where MWCNT were distributed, including proinflammatory cytokines and matrix remodeling enzymes. Molecular mechanism studies were performed in alveolar macrophages and lung fibroblasts to understand the relation between inflammation and fibrosis under MWCNT exposure, and the signaling pathways involved in both processes. Results from this study will contribute to both general concept on fibrogenesis and mechanism identification specific to MWCNT-initiated fibrosis.

3036 Characterization of a novel mouse model for spontaneous fibrosis

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We have established a novel mouse model, the NOD inflammation and fibrosis (N-IF) model, which spontaneously develops generalized inflammation and fibrosis at an early age. Due to a null mutation in the Rag2 gene, these mice do not produce any endogenous T or B cells. They do however produce a monoclonal NKT cell population due to the transgenic expression of a single 24αβ T cell receptor (TCR) isolated from a NKT-II clone. The N-IF mouse reveals an extensive fibrosis in multiple organs such as spleen, liver, skin and kidneys as well as a recruitment of mast cells and eosinophils in these organs. In line with this, a clear Th2 cytokine profile was evident both from the direct analysis of serum and after α-CD3 activation of spleen cells with high levels of IL-4, IL-5, IL-6 and IL-13. The inflammation can be transferred by spleen cells from affected animals and can be inhibited by splenic T cells derived from wild type NOD mice. We conclude that the N-IF mouse constitute a novel spontaneous model for fibrosis developing in association with chronic inflammation in multiple organs. We believe that this model provides a unique tool for analyzing the molecular mechanisms underlying fibrosis and for testing potential treatment protocols.

Fibrosis: From Bench to Bedside

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