Fibrosis: From Bench to Bedside

Scientific Organizers:
Jeremy S. Duffield | Steven R. Ledbetter | John P. Iredale

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3037 Novel murine heterotopic transplant model of intestinal fibrosis

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BACKGROUND: Severe mucosal tissue damage requiring efficient wound healing is a main feature of inflammatory bowel disease (IBD) but excessive tissue repair promotes fibrosis. The clinical investigation of fibrosis is confined to the limited amount of biological material available from patients. This makes the establishment of a new animal model a highly desirable goal. We investigated whether intestinal fibrosis occurs after heterotopic transplantation of small bowel resections in mice.

METHODS: Donor (B6) small bowel resections were transplanted subcutaneously into the neck of recipients (B6). Grafts were explanted 1, 2, 3, 4, 5, 6, 7, 14 and 21 days after transplantation.

RESULTS: Heterotopic intestinal transplants remained viable for 21 days. Rapid loss of crypt structures at day one after intestinal transplantation was followed by lymphocyte infiltration and obliteration of the intestinal lumen by fibrous tissue at day 21. Collagen expression was increased with time after transplantation as confirmed by real-time PCR, EvG and Sirius Red staining. Lumen obliteration was connected with increased expression of potent mediators of fibrosis such as TGF-β.

CONCLUSION: We established a method for heterotopic transplantation of small bowel resections in mice. A variety of histologic and molecular features of fibrosis were observed in the heterotopic intestinal grafts which suggests, that this new *in vivo* model will be instrumental in studying pathogenesis and treatment of intestinal fibrosis.

3038 Intestinal organoids: A model of intestinal fibrosis

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Purpose: To develop a multi-cellular, 3-dimensional organoid model of intestinal fibrosis.

Background: Intestinal fibrosis is a critical complication of Crohn's disease. Current *in vitro* intestinal myofibroblast models of fibrosis which are activated by cytokine stimulation (e.g. TGFβ) or the physical environment (mechanical stress) do not fully recapitulate the complex multicellular 3-dimensional (3D) intestinal architecture while *in vivo* models (rat TNBS, mouse (*S. typhimurium*) have limited utility for large-scale screening of anti-fibrotic compounds or analyzing the mechanistic contributions of multiple genetic loci. Here, we exploit recent advances in 3D *in vitro* growth of intestinal tissue as a new human model to study intestinal fibrosis associated with IBD.

Materials and Methods: Human pluripotent stem cells were differentiated into human intestinal organoids (HIOs), which are 3D structures comprised of a lumen, intestinal epithelium and mesenchyme. In order to identify myofibroblasts, which are key effectors of fibrosis, 45-day old HIOs were stained for a-smooth muscle actin (αSMA), vimentin, and desmin by immunofluorescence. TGFβ responsiveness was assayed by treatment of organoids with increasing amounts of TGFβ for 48 hrs or 2 ng/ml of TGFβ for 96 hr. Fibrotic response was assayed by expression of pro-fibrotic genes (col1A1, ACTA2, Fn1, MYLK, and MKL1).

Results: Immunofluorescent staining of organoids from healthy controls identified a population of myofibroblasts (αSMA+/vimentin+/desmin- cells), which-11 were identified as a discrete population of cells within the intestinal mesenchyme, located adjacent to the intestinal epithelium. Healthy control organoids were TGFβ-responsive as increasing concentrations of TGFβ for 48hr induced a dose-dependent pro-fibrotic response with increased Fn1 and MKL1 gene expression. Longer exposure (96 hr) to TGFβ (2 ng/ml) triggered a more robust fibrotic response with significant 2 to 4-fold increases observed in col1A1, ACTA2, MYLK, Fn1, and MKL1 gene expression.

Conclusions: Intestinal organoids derived from healthy tissue develop a myofibroblast layer and respond to pro-fibrotic stimuli, in a manner that is consistent with myofibroblasts isolated from normal and IBD patients. Given that our current understanding of the etiology of fibrosis in IBD patients is severely lacking, generation and use of HIOs from both healthy and IBD patients will enable elucidation of the cellular, molecular and genetic differences between the healthy and IBD states. HIOs could also advance the screening of anti-fibrotic therapies for Crohn's disease.

3039 Multi-walled carbon nanotubes induce proinflammatory cytokine expression by activating NF-κB signaling in mouse lungs

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Occupational and environmental exposure to respirable particles and fibers potentially causes lung fibrosis that is often progressive and non-curable in humans. The mechanism by which fibrogenic particulates and fibers induce pulmonary fibrosis is largely unknown. Multi-walled carbon nanotubes (MWCNT) are fiber-like, new materials of graphitic carbon with diameters in the nanometer range, and variable aspect ratios and surface chemistry, which render unique electronic, mechanical, and chemical properties. The health risk that MWCNT may impose on humans is unclear. MWCNT induce rapid-onset and progressive fibrosis in mouse lungs that is accompanied by significant inflammatory infiltration and cytokine expression. In this study, induction of gene expression was exploited to uncover the signaling pathways governing the inflammatory and fibrotic responses to MWCNT. MWCNT stimulated macrophages to transcribe and secrete proinflammatory cytokines including IL-1β, TNFα, IL-6, and MCP1. Moreover, MWCNT activated the NF-κB signaling pathway both in vivo and in cultured macrophages, evidenced by MWCNT-induced degradation of IκBα, nuclear accumulation of NF-κB p65, and binding of activated NF-κB to a consensus NF-κB DNA binding sequence. MWCNT stimulated the expression of a reporter gene under the control of either 5 copies of a NF-κB binding sequence or the mouse TNFα gene promoter that contains multiple copies of the NF-κB binding elements. Lastly, culture media from macrophages exposed to MWCNT were found capable of activating fibroblasts to transform into myofibroblasts. Current studies are undertaken to elucidate the molecular events by which MWCNT trigger NF-κB activation.

3040 Human and animal tissue slices for the development of anti-fibrotic compounds

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To improve and accelerate anti-fibrotic drug discovery, there is an urgent need for reliable and reproducible (human) in vitro methods to test compounds for the treatment of fibrosis. The current in vitro models used cannot predict the complex cellular interactions that occur in vivo and lack the cellular diversity that epitomize specific organs. And although organs share major common disease pathways, for immune cells and especially myofibroblasts, the main extracellular matrix producing cells, there are organ- and etiology-specific variations in cellular gene expression profiles. Therefore, it is of utmost importance to explore the pathophysiological mechanism of fibrosis across the different organs. Consequently, ex vivo models are needed that resemble the in vivo (human) environment. Precision-cut tissue slices (PCTS) represent an ex vivo tissue culture technique that replicates most of the multicellular characteristics of whole organs in vivo. Moreover, PCTS is ideal for studying organ differences, since PCTS can be prepared from most organs, while the cells, e.g. the myofibroblasts, are retained in their original environment.

In our lab, PCTS were successfully prepared from rodent and human livers, intestines and kidneys. Most organ PCTS were viable up to 48 hours, as demonstrated by the ATP content of the slices. After 48 hours of culturing the onset of fibrogenesis, as determined by the expression of multiple gene markers of fibrosis, including Pro-Collagen 1 A1 (PCOL1A1), Heat Shock Protein 47, alpha-Smooth Muscle Actin, was induced spontaneously in almost all organ PCTS. Moreover, incubation of PCTS with TGF-beta induced the expression of PCOL1A1 in most organ slices.

Thus, rodent and human PCTS can be used to study the onset of fibrosis and are promising models for testing the efficacy of anti-fibrotic compounds.