347. Stem cells and progenitors in injury and repair

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LSC 2012 Abstract – Effects of stem cells of healthy or acute lung injury donors of recipient injury mice

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We aimed to investigate the effects of BMDMC from healthy and ALI donors in experimental model of ALI. For this, 55 female C57BL/6 mice were randomly assigned into four groups. The control groups received saline. ALI mice received E.coli LPS intratracheally (ALIp) or intraperitoneally (ALIexp). After 24h, 5x106 whole BMDMC from all groups were subjected to in vitro colony forming units-fibroblastoid (CFU-F) and flow cytometry. After cell characterization, all animals were treated with saline or BMDMC (i.v.) obtained from healthy and ALIp and ALIexp donors at 24h. In ALIp, CFU-F assay showed a predominance of non-stromal cells over fibroblastoid colony. In ALIexp, an irregular CFU-F morphology was observed. In ALIp, monocytes and T lymphocytes were increased and hematopoietic precursor cells reduced. At day 7, mortality rate was higher in ALI groups, and after BMDMC therapy reduced. BMDMCs attenuated lung mechanics, neutrophils, alveolar collapse, as well as fibers content. Additionally, reduced the levels of citokynes in lung tissue independent of cell origin. BMDMCs reduced the inflammatory and fibrogenic processes, improving lung mechanics; nevertheless, BMDMCs from ALI animals were less effective at reducing the inflammatory process compared to those originated from healthy donors.

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Clara cells serve as the progenitors to regenerate alveolar epithelium in response to severe lung injury

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Bleomycin treatment or influenza virus infection can induce severe damage in distal lung with the loss of large amounts of alveolar type II (AT2) and type I (AT1) cells. During the repair process, new AT2s and AT1s have to be produced to regenerate the damaged alveoli. A recent study showed that the newly generated AT2s were not derived from pre-existing AT2s after bleomycin treatment, indicating the existence of other progenitor cells for alveolar regeneration [1]. We have used a genetic lineage tracing system to follow Clara cells in mice. We show that large numbers of the newly generated AT2s and AT1s are derived from Clara cells after alveoli damage by bleomycin treatment or influenza virus infection. The intermediates between Clara cells and AT2s are SPC-expressing bronchiolar cells (or SBECs). SBECs are only observed in damaged area and initially positive for Clara cell marker Clara Cell Secretory Protein (CCSP) and gradually become CCSP negative. Anatomical analysis shows that SBECs at the tips of bronchioles appear to dilate to regenerate alveolar epithelium in a process similar to that seen during the development of embryonic alveolar epithelium. These findings show that Clara cells are the progenitors to regenerate alveolar epithelium in response to severe lung damage.

Reference: [1] Chapman, H. A. et al. Integrin alpha6beta4 identifies an adult distal lung epithelial population with regenerative potential in mice. J Clin Invest 121, 2855-2862 (2011).

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Secreted mediators from induced pluripotent stem cells (iPSc) attenuate fibrosis in bleomycin injured rat lung

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Background Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease resulting from deregulated alveolar epithelial repair, after micro injuries. There are no promising treatments available hence; novel methods to regenerate the injured lungs are urgently required. We studied the role of secreted mediators from induced pluripotent stem cells (iPSc) in bleomycin injured rat lungs.

Methods: iPSc cells were generated from human foreskin fibroblasts by transfection of the transcription factors SOX2,OCT4, KLF4, and c-MYC; after characterization by immunohistochemistry and RT PCR, the colonies were expanded and the conditioned media (CM) was collected (iPScCM). iPScCM was subjected to proteomics to analyze the contents. Adult male rats (F344) were instilled intratracheally (i/t) with bleomycin at day 0; 7 days later were treated with iPScCM or control media (CM) (i/t) and sacrificed 7 days after iPScCM or CM treatment.

Results: Proteomic analysis revealed presence of various interesting cytokines and growth factors in the iPScCM, which are involved in regeneration process; the total collagen content after iPScCM treatment was reduced compared to CM group (753±56.12 ug/mg vs 4182±521.8 ug/mg of wet tissue) as measured by hydroxyproline assay. Furthermore, TGFβ1 mRNA levels were also reduced after iPScCM treatment (0.9±0.266 vs 2.4±0.9) (2-ΔΔCP).

Conclusion: Secreted mediators present in the iPScCM attenuate fibrosis in the bleomycin injured rat lungs and may offer a novel therapeutic option for pulmonary fibrosis.

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Notch signaling via hairy/enhancer of Split-5 (Hes5) & paired-box containing gene 6 (Pax6) controls progenitor cell reservoir for repair of airway injury

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Background: The airways of the mammalian lung are lined with specialized epithelial cell types that are the target of airborne toxicants and injury. Notch signaling plays an important role in the ontogeny of these cells, but its contributions to recruitment, expansion or differentiation of resident progenitor/stem cells in repair of injured airways remains unknown.

Aims & objective: To elucidate the role of Notch signaling in repair of injured airway epithelium.

Methods: We used targeted inactivation of, Notch1, via the epithelial-specific Gata5-cre line in the embryonic lung epithelium.

Results: Notch1(-/-) mice are viable with intact pulmonary epithelial cell fate determination/differentiation. However, Notch1 was found to be required for normal repair of the injured airway epithelium. Absence of Notch1 reduced the ability of cells distinguished by expression of PGP9.5, a marker of pulmonary neuroendocrine cells, which serve as a reservoir for regeneration of Clara cells.

Hairy/Enhancer of Split-5 (Hes5) and a paired-box containing gene 6 (Pax6) were found to be downstream targets of Notch1. Both Hes5 and Pax6 expression were significantly increased in association with Clara cell regeneration in wild type lungs. Ablation of Notch1 reduced Hes5 and Pax6 and inhibited airway epithelial repair. Thus, although dispensable in developmental ontogeny of airway epithelial cells, normal activity of Notch1 is required for repair of the airway epithelium. The signaling pathway by which Notch1 regulates the repair process also includes stimulation of Hes5 and Pax6 gene expression.
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Evidence of increased pluripotent cells in adult human lung tissue derived from fibrotic lungs compared to non-fibrotic control lungs
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Introduction: Tissue-specific multipotent stem cells have been identified in the human lung. However, their role in lung homeostasis or lung disease is not clear.
Methods: Primary human lung cells were cultured from fibrotic adult lung parenchyma (n=14) and from non-fibrotic control lungs (n=17). The characterization of different cell types was performed by immunofluorescence stainings.
Results: Undifferentiated primary cells grew from adult human lung parenchyma, showing neither a clear epithelial nor mesenchymal morphology/immunofluorescence typing (=intermediate cells). When cultured in the respective appropriate media, intermediate cells transformed into mesenchymal cells (positive for fibronectin and α–smooth muscle actin) or into alveolar epithelial type II cells (positive for E-cadherin and surfactant protein-A). Pluripotency of intermediate cells was proven by positive stainings for Oct3/4 and NANOG. Successful induction of adipogenic, osteogenic, myogenic, and chondrogenic differentiation was performed in intermediate cells. Finally, significantly more pluripotent cells were generated from fibrotic lung tissue (n=14) than from non-fibrotic controls lungs (n=17).
Conclusions: Our data demonstrate that adult human lung contains pluripotent cells which are able to differentiate towards an epithelial as well as a mesenchymal cell type solely by changing their microenvironment. These pluripotent cells might have a pivotal function in lung homeostasis and tissue repair. The observed increased incidence of these cells in fibrotic lung tissue suggests a role in fibrogenesis.

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Adrenaline stimulated mesenchymal stem cells modulate inflammation in lipopolysaccharide induced lung injury
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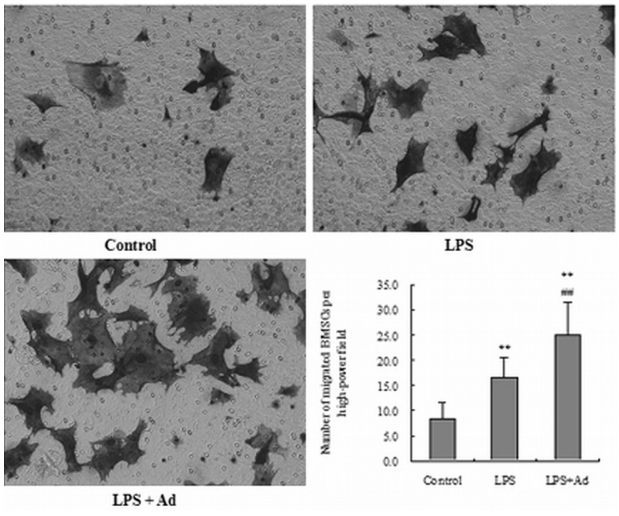
Introduction: Bone marrow derived mesenchymal stem cells (BMSCs) could modulate inflammation. Adrenergic receptor agonists could increase DNA synthesis and protect oxidative stress in mesenchymal stem cells. We investigated the potential role of adrenaline stimulated BMSCs on lipopolysaccharide (LPS) induced lung injury in vitro.
Methods: BMSCs from rats were cultured with adrenaline at 0-100μM, followed by determination of CCK8. The optimal concentration was chosen for latter study. BMSCs and lung cells from normal or LPS injured rats were co-cultured in a Transwell system (8μm pore size) for 36h. The migrated BMSCs were stained by Giemsa. BMSCs and alveolar macrophages were co-cultured with LPS or adrenaline in Transwell (0.4μm pore size) for 6h. The supernatant was collected for cytokines (TNF-α, IL-1beta, IL-6, IL-10, IL-13, angiopoietin-1, KGF and IL-1ra) analysis by ELISA, and the mRNA expression levels in BMSCs were determined by PCR.
Results: Adrenaline at 10μM promoted proliferation and migration of BMSCs towards injured lung. Adrenaline could enhance the inflammation modulation effects of BMSCs by decreasing TNF-α, IL-1beta and IL-6, and increasing IL-10. Adrenaline could also increase angiopoietin-1 mRNA expression of BMSCs.
Conclusions: Adrenaline could help BMSCs modulate LPS induced lung injury in vitro, probably through promotion of proliferation, migration and angiopoitein-1 secretion.



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LSC 2012 Abstract – Mesenchymal stromal cells identified in tissue from lung-transplanted patients
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Background: Chronic rejection expressed as bronchiolitis obliterans (BO) is seen in 50% of lung-transplanted (LT) patients. BO is characterized by extra cellular matrix deposition, where the fibroblast is thought to be a key player. The fibroblast origin is at present not fully known. The fibrocyte is one of the potential origins, and our group has shown that fibrocytes are associated with remodelling in BO. In this study we focused on another origin the mesenchymal stromal cell (MSC).
Objectives: Our aims were to examine whether MSC are present in tissue from LT patients. We wanted to evaluate whether these cells displays characteristic MSC properties such as adherent clonal growth, multi-lineage potential and a characteristic surface marker profile.
Methods: MSC were isolated from biopsies of LT patients and the single cell suspension was seeded in colony-forming unit-fibroblast (CFU-F) assays. Isolated MSC were differentiated towards adipocytes, osteoblasts and chondrocytes.Their surface markers was examined by flow cytometry.
Results: MSC isolated from LT patients formed typical colonies, displayed spindle-shaped morphology and adhered to plastic. Furthermore, cells displayed multi-lineage potential. Analysis of the surface markers showed that MSC showed expression of CD73, CD105 and CD90, but lacked expression of CD45 and CD34.
Conclusions: Our study suggests that MSC are present in lung tissue from LT patients. These cells display colony growth, multipotential and a characteristic MSC surface marker profile.

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LSC 2012 Abstract – TGF-β1 regulates the epithelial supportive capacity of mesenchymal stromal cells
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Despite recent advances in biomarker profiling, prospective isolation and clonogenic assay of putative lung stem cells their regenerative capacity remains ill-defined. On that account we have developed a clonogenic colony-forming assay that has enabled us to identify a population of multi-potent lung epithelial stem cells (EpCAMhi CD49fpos CD104pos CD24low) that are able to self-renew and give rise to airway and alveolar epithelial cell lineages. However,the intrinsic regenerative potential of stem cells is conditional upon their interaction with permissive and restrictive microenvironmental cues. On this note, we have shown that the proliferation and differentiation of lung epithelial stem cells in vitro is dependent on co-culture with endogenous lung mesenchymal stromal cells (EpCAMneg Sca-1pos), or mesenchyme-derived growth factors including FGF-10 and HGF. On the flip side, we have shown that more differentiated mesenchymal stromal cells (FGF-10low α-SMApos) are unable to support epithelial colony formation. Importantly, we have shown that the capacity of mesenchymal stromal cells to support epithelial stem cells is regulated by TGF-β1 and can be reversed by blockade of SMAD2/3 phosphorylation downstream of TGF-β activation. This data suggests that TGF-β mediated mesenchymal differentriation in chronic lung diseases may obstruct epithelial regeneration.