Effect of Hyperoxia in Mouse Lung Fibroblasts

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Hyperoxia causes considerable lung injury and mortality, and diffuse epithelial injury and proliferation of fibroblasts are considered as most important cellular mechanism. We aimed to investigate the effect of hyperoxia on mouse lung fibroblasts (MLF). Primary MLFs were isolated from C57BL/6 mice or OTO mice, in which GFP for α-smooth muscle actin (α-SMA) protein is over-expressed. MLFs were exposed to >90% O2 in a Plexiglass chamber for 24, 48, or 72 hours. As comparison, MLFs exposed to normoxia were used. We also placed MLFs to normoxia for 24, 48 or 72 hours after 48 hours of hyperoxia for recovery. Total cell count with viability, western blots for IGF-R and α-SMA, quantitative RT-PCR for IGF-R, α-SMA, Collagen 1 α, and CXCL-1 were done. GFP expression for α-SMA was measured in MLFs from OTO mice. Collagen gel contraction assay, microscopic evaluation for MLFs morphology and senescence associated (SA)-β-galactosidase (gal) stain were also done. As results, total cell counts were lower in hyperoxia-exposed MLFs without any significant difference in viability compared to normoxia control. MLFs exposed to hyperoxia showed larger, flattened appearance with increased SA-β-gal stain. Hyperoxia decreased the level of α-SMA and Collagen 1 α. Hyperoxia decreased IGF1R expression and increased the level of CXCL-1. Collagen gel contraction was decreased with hyperoxia. In summary, hyperoxia induced cellular change compatible with cellular senescence in MLFs. Hyperoxia also decreased the level of fibroblasts activation markers and contractility.

The Role of Transglutaminase-2 in Ventilator-associated Lung Injury

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Background: Transglutaminase-2(TG-2) has been reported to play an important role in the process of inflammation. The purpose of this study is to investigate the role of TG-2 in the pathogenesis of ventilator-associated lung injury (VALI).

Methods: 5 weeks of age, male C57BL/6 mice were used. VALI was induced by mechanical ventilation (tidal volume 35 mL/kg+respiratory rate 90/min+PEEP 0 cmH2O+4 hours) after lipopolysaccharide(LPS, 0.5mg/Kg/50uL i.t.) instillation. The mice were experimentally divided into five groups: control, LPS, VALI, LPS+Cyst (LPS+cystamine, TG-2 inhibitor, 200 mg/kg i.p.) and VALI+Cyst groups. TG-2 activities were measured in the lung tissue homogenates using fluorescence based protein arrays. Inflammatory cytokines, myeloperoxidase (MPO) and nuclear factor-κ B (NF-κ B) activity were determined.

Results: The TG-2 activities in the control, LPS, VALI, LPS+Cyst and VALI+Cyst groups were 141.3±6.9, 177.7±5.6, 202.6±4.2, 162.9±9.9 and 155.5±5.0 nmol/min/mL, respectively (p=0.001 by Kruskal-Wallis test), in which the VALI+Cyst group showed significantly lower TG-2 activity than the VALI group (p=0.001). Tumor necrosis factor-α, interleukin (IL)-1β, and IL-6 concentrations were significantly lower in the VALI+Cyst group than the VALI group (p<0.05). NF-κ B activity of the VALI+Cyst group were also decreased than the VALI group (p=0.017).

Conclusion: TG-2 activity was increased in VALI. The treatment with TG-2 inhibitor decreased the TG-2 activity, inflammatory cytokines and NF-κ B activity, suggesting potential role of TG-2 in the pathogenesis of VALI.