Alcohol induces profibrotic fibroblast phenotype via induction of DNA methyl transferase.
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BACKGROUND: We previously determined that alcohol induces fibroproliferative disrepair following bleomycin-induced acute lung injury through induction of TGFβ1 expression. We further showed that alcohol suppressed cellular anti-oxidant response through suppression of Nrf2 expression and treatment with Nrf2 activator sulforaphane (SFP) attenuated the effect of alcohol on lung fibroblasts including normalization of TGFβ1 expression. In follow up studies, we have shown TGFβ1-mediated lung fibroblast Thy-1 expression through induction of Thy-1 promoter methylation. Loss of Thy-1 has been shown to be associated with development of fibrosis in experimental and clinical models. Therefore, we hypothesized that alcohol primes the lung toward fibroproliferative disrepair following acute injury through TGFβ1-mediated Thy-1 promoter methylation, leading to a decrease in lung fibroblast Thy-1 expression. We further hypothesize that SFP attenuates the effects of alcohol on lung fibroblasts through epigenetic mechanisms.

METHODS: Mouse primary lung fibroblasts (PLF) were cultured ± alcohol (60 mM) ± ALK5 inhibitor (8 μM) ± SFP (5 μM) for 48 hrs at which time Thy-1, DNMT1, DNMT3a, and DNMT3b gene expression, DNMT activity, and Thy-1 promoter methylation by qMethyl PCR were determined. In selected experiments, Thy-1 protein expression was quantified after 72 hrs of culture in alcohol. In parallel, the lungs from alcohol-fed mice (20% of total calories in water) for 6 weeks ± SFP (5 mg/kg/day IP, 3 days a week) for the last 2 weeks were analyzed for DNMT1 gene expression.

RESULTS: Alcohol treatment suppressed Thy-1 gene and protein expression while induced DNMT1 and DNMT3b gene expression, DNMT activity, and Thy-1 promoter methylation. Inhibition of TGFβ1 signaling with ALK5 inhibitor or treatment with SFP (which we previously shown to attenuate alcohol-induced TGFβ1 expression) restored Thy-1 gene expression and attenuated alcohol-induced DNMT activity and Thy-1 promoter methylation. Lastly, chronic alcohol exposure induces DNMT1 gene expression in the whole lung tissue while treatment with SFP suppressed it.

CONCLUSIONS: This study elucidates a potential mechanism by which alcohol primes the lung toward fibroproliferative disrepair following acute injury through induces loss of Thy-1 expression by lung fibroblasts. The data shown here suggested that alcohol-induced TGFβ1 which lead to induction of DNMT1 expression, DNMT activity and Thy-1 promoter methylation. Treatment with SFP seems to mitigate the effect of alcohol on lung fibroblasts, however, the mechanism of action is to be further investigated.

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