CYTOKINES IMPACT FIBROBLAST ACTIVITY AND TUMOR FIBROSIS IN PANCREATIC DUCTAL ADENOCARCINOMA

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**Background** Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease. The five-year survival is only 11%. One hallmark of PDAC is the excessive fibrotic reaction in the tumor microenvironment surrounding the tumor cells. Tumor fibrosis is characterized by an increased activity of fibroblasts and turnover of collagens. Unfortunately, this leads to reduced drug-uptake, impaired immune cell activity and as a result reduced survival in patients. Cytokines in clinical cancer immunotherapy, either as monotherapy or in combination with check-point inhibitors have gained much attention as anti-cancer drugs. Some cytokines have been shown to possess pro- and anti-fibrotic properties and could therefore provide additive value to the anti-cancer efficacy. TGF-β, PDGF-AB, IL-1α, IL-1β, IL-8 and IL-15, are six cytokines highly investigated in clinical trials as monotherapies or in combination with immunotherapies. In this study, we investigated the pro- and anti-fibrotic effects of these cytokines in molecular crowding pancreatic fibroblast culture assays in combination with collagen biomarkers measuring formation of type I, III and VI collagen (PRO-C1, PRO-C3 and PRO-C6, respectively).

**Methods** Human pancreatic fibroblasts were cultured in Ficoll-media. Fibroblasts were treated w/wo TGF-β (1ng/mL), PDGF-AB (100 ng/mL), IL-1α (10 ng/mL), IL-1β (10 ng/mL), IL-8 (10 ng/mL) and IL-15 (10 ng/mL). The fibrotic activity of the fibroblasts was investigated by measuring PRO-C1, PRO-C3 and PRO-C6 in the supernatant at days 3, 6, 9 and 12. Cell-viability was evaluated by Alamar-blue.

**Results** The formation of type I collagen (PRO-C1) was significantly increased when fibroblasts were treated with TGF-β, IL-1α and IL-1β, but not PDGF, IL-8 and IL-15 at days 6, 9 and 12. The formation of type III collagen (PRO-C3) was significantly increased when fibroblasts were treated with TGF-β, IL-1α and IL-1β, but not PDGF, IL-8 and IL-15 at days 6, 9, compared to no treatment. At day 12, PRO-C3 was significantly increased in supernatant from fibroblasts treated with TGF-β, IL-1α, IL-1β and IL-8, but not IL-15, compared to no treatment. Interestingly, the formation of type VI collagen (PRO-C6) was only increased when fibroblasts were treated with PDGF-AB compared to no treatment at days 3, 6, 9 and 12. There were no differences in cell viability between treatments.

**Conclusions** TGF-β, PDGF-AB, IL-1α, IL-1β, IL-8 and IL-15 have differential effects on the pro-fibrotic activity of pancreatic fibroblasts. This is important knowledge when developing cytokine drugs for clinical cancer immunotherapy trials, suggesting that tumor fibrosis needs to be accounted for when designing and evaluating such studies.