COLLAGEN IS NOT JUST COLLAGEN – DIFFERENTIAL MATRIX EXPRESSION INDUCED BY TGF-β AND PDGF

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Background and aim

Accumulation of extracellular matrix (ECM) proteins is a hallmark of fibrosis, which can lead to altered tissue homeostasis, organ failure and ultimately death. Many different cell types and growth factors are involved in this process, but fibroblasts are the main source of ECM proteins. With the aim of investigating the effects of tumor growth factor (TGF)-β and platelet-derived growth factor (PDGF)-AB and BB induced synthesis of different ECM proteins, we here present results from an in vitro model, using human fibroblasts.

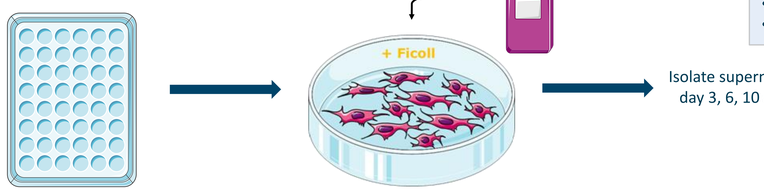
Fibroblast stimulation with TGF-β or PDGF-AB or -BB

Induction of fibrogenesis

Methods

The effect of TGF-β and PDGFs on ECM protein synthesis was assessed in a scar-in-a-jar (SiaJ) cell model using human fibroblasts. Cells were seeded in 48-well plates at 30.000 cells/well and incubated for 24H in DMEM + 10% FBS for adherence. Serum starvation was done by seeding the cells for further 24H in DMEM + 0.4% FBS. Fresh medium was added at day 0 with 225/150mg/mL Ficoll 70/400 and 1% ascorbic acid, containing 0.04 nM TGF-β, 3.9-, 0.39-, or 0.039 nM PDGF-AB or -BB or a vehicle control. Medium was changed and collected at day 3, 6, 10 and 13. Biomarkers of collagen type I (PINP), III (PRO-C3), VI (PRO-C6) and fibronectin (FBN-C) formation were assessed in the medium by ELISAs developed at Nordic Bioscience.

3D culturing of fibroblasts

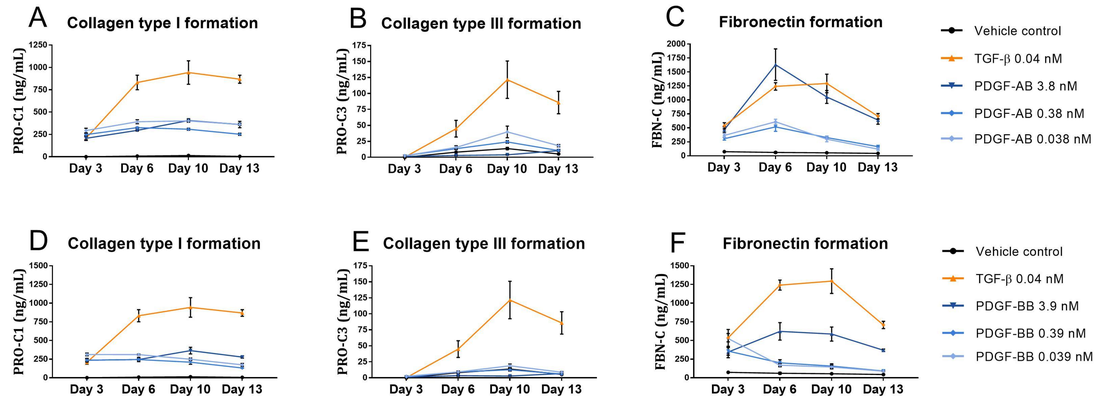


Treatments
- Vehicle control
- TGF-β
- PDGF-AB
- PDGF-BB
- w/o Ficoll, w. TGF-β

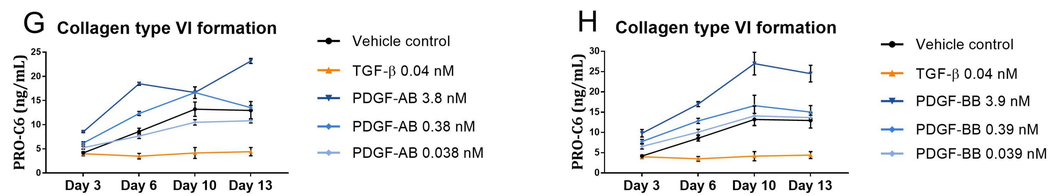
Conclusions

Different growth factors induce different protein expression profiles in fibroblasts. Collagen synthesis is thus regulated differentially. This SiaJ model in combination with the investigated biomarkers of ECM formation could be used to elucidate the mechanisms behind acute and sustained ECM production profiles. This model setup applies to different diseases were fibroblasts play a role, including liver fibrosis.

Results



Stimulating fibroblasts with TGF-β significantly increased the formation of type I (A) and III collagen (B) as well as Fibronectin (C) when compared to unstimulated cells. The increase in formation peaked at day 10 for all markers, with a more than 50-fold increase for type I collagen and Fibronectin, and a 10-fold increase for type III collagen formation.
Both PDGF-AB and -BB increased levels compared to untreated cells in a dose-dependent manner (A-F). However, the highest concentration of PDGF-AB (3.8 nM) was found to induced a significant increase in the levels of Fibronectin at day 6 (C).



TGF-β treatment did not enhance synthesis of type VI collagen at any measured time point, but suppressed the formation compared to the untreated cells (G-H). Higher concentrations of PDGF-AB and -BB did, however, increase synthesis of type VI collagen compared to untreated cells (G-H). 3.8 nM PDGF-AB induced type VI collagen levels 2.15-fold at day 6, and 0.38 nM PDGF-AB induced synthesis in an increasing manner from experiment start to end (G).

A dose-dependent increase in type VI collagen levels was observed with PDGF-BB treatment (H). Levels peaked at day 10 after treatment with 3.9 nM PDGF-BB (2-fold increase compared to untreated cells).

EASL The Home of Hepatology | THE INTERNATIONAL LIVER CONGRESS™ APRIL 10 - 14, VIENNA, AUSTRIA 2019

NORDIC BIOSCIENCE

Disclosures: Villesen, Reese-Petersen, Karsdal and Genovese are employees of Nordic Bioscience.

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DOI: 10.3252/pso.eu.ILC2019.2019

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THU-099
ILC2019