Unraveling Collagen Signatures in Cancer-Associated Fibroblasts: A Biomarker-driven Approach

Annika Hettich[1,2], Neel Ingemann Nissen[1], Morten Karsdal[1], Nicholas Willumsen[1]
[1]Nordic Bioscience, Herlev, Denmark
[2]Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

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BACKGROUND

The tumor microenvironment (TME) plays a crucial role in driving tumor development. Among the constituents of the tumor stroma, cancer-associated fibroblasts (CAFs) are a pivotal component. CAFs are actively involved in tumor progression by modulating the architecture of the TME through increased deposition of various collagens resulting in tumor fibrosis. Several studies have shown that CAFs have heterogeneity within, and between, individual tissues. TGF-β is thought to be the main driver of tumor fibrosis, however, the field lacks a characterization of the specific collagen deposition of CAFs from different tissues.

In this study, we investigated the fibrotic activity of CAFs from various tissues by measuring the production of three specific collagen peptides *in vitro* by use of non-invasive clinically validated biomarkers.

METHODS

Primary human CAFs from pancreas (pCAF), breast (bCAF), colon (cCAF) and lung (lCAF) were cultured over a 12-day period in ficoll-based media under both untreated conditions and TGF-β (10 ng/ml) stimulation. Additionally, cells were subjected to treatment with the ALK5/TGF-β1 receptor kinase inhibitor (ALK5i, 1.9 µM). The assessment of type I collagen (PRO-C1), type III collagen (PRO-C3), and type VI collagen (PRO-C6) formation was conducted in the cell supernatant from day 3, 6, 9 and 12 using competitive enzyme-linked immunosorbent assay (ELISA). On day 12, the matrices were decellularized and stained with Sirius red to visualize the net-collagen content.

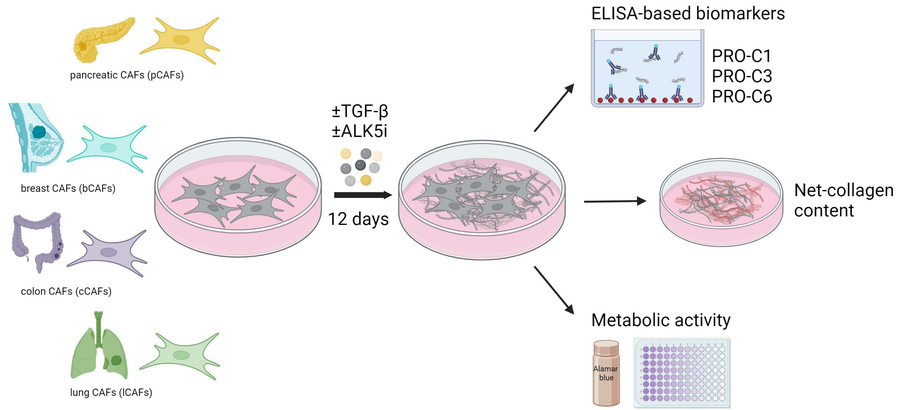

Figure 1: pCAFs, bCAFs, cCAFs, and lCAFs were cultured for 12 days in a ficoll-based media. Cells were either untreated, stimulated with TGF-β alone, or additionally treated with ALK5i. At the end of each experiment the biomarkers PRO-C1, PRO-C3, and PRO-C6 were measured in the cell supernatants; the presence of deposited collagen was visualized using Sirius red; and the metabolic activity of the cells was assessed using Alamar blue. Created with BioRender.com

Contact: Annika Hettich, anhe@nordicbio.com
Disclosures: NWI and MK are employed at Nordic Bioscience and may be shareholders.

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RESULTS

Collagen Signature of Different CAFs

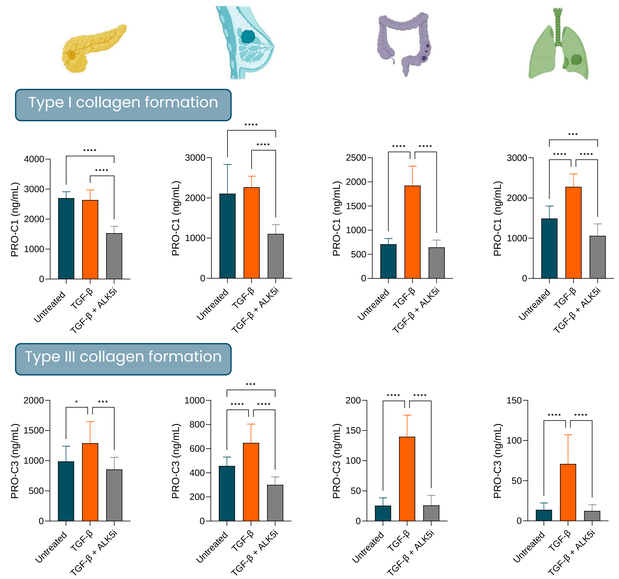

Figure 2: pCAFs, bCAFs, cCAFs and bCAFs show a distinct collagen signature. The biomarkers PRO-C1 (type I collagen formation), PRO-C3 (type III collagen formation), and PRO-C6 (type VI collagen formation), were measured in the supernatant of untreated CAFs or CAFs stimulated with TGF-β with or without ALK5i at day 3, 6, 9, and 12. Shown is the accumulated data of all four days. n = 3-4 technical replicates and 4 biological replicates.

Net-Collagen Content

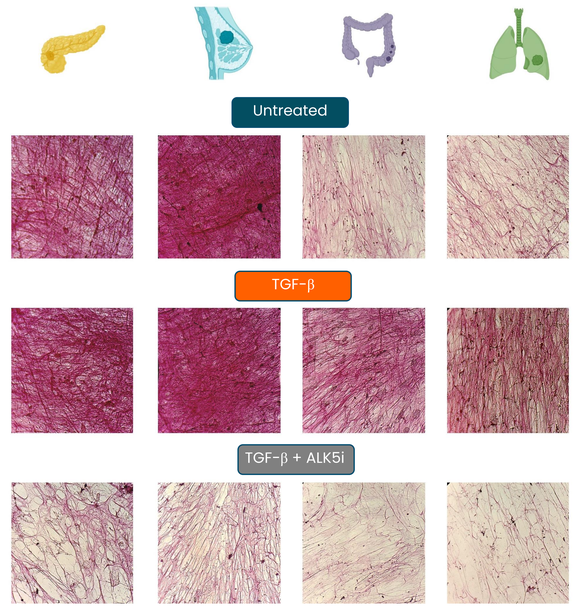

Figure 3: The collagen signature of different CAFs is reflected in the overall net-collagen content. At day 12 the ECM of the different CAFs was decellularised and the deposited collagen was visualized using Sirius red staining. Pictures are at x20 magnification.

Metabolic Activity

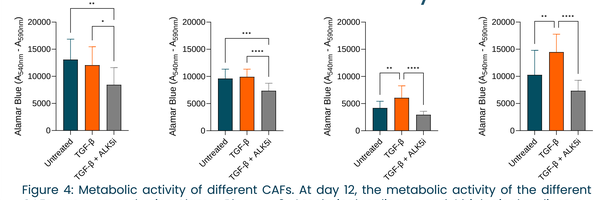

Figure 4: Metabolic activity of different CAFs. At day 12, the metabolic activity of the different CAFs was assessed using Alamar Blue. n = 3-4 technical replicates and 4 biological replicates.

* p<0.05 ; ** p<0.01; *** p<0.001; **** p<0.0001

CONCLUSION

- These findings underscore the heterogeneity in collagen production among CAFs from different indications, providing valuable insights into the ECM dynamics within distinct TMEs.
- Collagen-based non-invasive biomarkers demonstrate the capability to differentiate between the fibrotic activity of CAFs isolated from different tissues
- This model proves to be a useful tool for anti-fibrotic drug screening