Serological assessment of cancer associated myo-fibroblast (myCAF) activity by collagen pro-peptide biomarkers provides high prognostic power

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BACKGROUND

- Myofibroblast Cancer Associated Fibroblasts (myCAFs) are the main tumor fibrosis drivers and hence different from inflammatory CAFs (iCAFs).
- MyCAFs are important for understanding tumor biology.
- CAFs produce type III, V, VI and XI collagen that are the essential components of tumor fibrosis.
- Pro-peptides of these collagens can be quantified both in serum with the PRO-C3, PRO-C5, PRO-C6 and PRO-C11 biomarkers where they are prognostic for poor overall survival in patients with various solid tumor types and may be applied in vitro.
- Here we investigated the association and difference between myCAFs and iCAFs and their collagen expression profile and related that to data available data on serological assessments of PRO-C3, PRO-C5, PRO-C6 and PRO-C11, and cultured CAFs.

METHODS

- The collagen expression in CAF subtypes were established from publicly available single-cell RNA-Seq dataset from pancreas cancer (PDAC, PMID: 31273297) and non-small cell lung cancer (NSCLC, PMID: 36973297).
- PRO-C3, PRO-C5, PRO-C6 and PRO-C11 ELISAs to measure serum of 220 patients with various solid tumor types and 33 healthy controls (Cohort 1).
- CAFs and NFs was cultured in vitro to measure biomarkers in conditioned medium.
- Historical PRO-C3, PRO-C5, PRO-C6 and PRO-C11 prognostic data for pancreas cancer patient data was included for comparison (the BIOPAC cohort).

Cohort 1:

Diagnosis	n	Age, Mean (SD)	Sex F/M, n	Stage I,II,III,IV, n
Healthy	33	58 (6)	12/21	-
Bladder cancer	20	65 (11)	5/15	0,9,11,0
Breast cancer	20	53 (10)	20/0	1,16,3,0
Colorectal cancer	20	55 (10)	7/13	0,4,8,8
Head & Neck cancer	20	55 (9)	9/11	0,0,4,16
Kidney cancer	20	56 (10)	10/10	5,1,8,6
Lung cancer	20	61 (7)	3/17	0,0,14,6
Melanoma	20	60 (16)	12/8	1,4,9,6
Ovarian cancer	20	56 (12)	20/0	0,0,13,7
Pancreatic cancer	20	66 (7)	10/10	0,11,2,7
Prostate cancer	20	65 (5)	0/20	0,1,14,5
Gastric cancer	20	63 (20)	5/15	0,0,7,13

BIOPAC (NCT03311776):

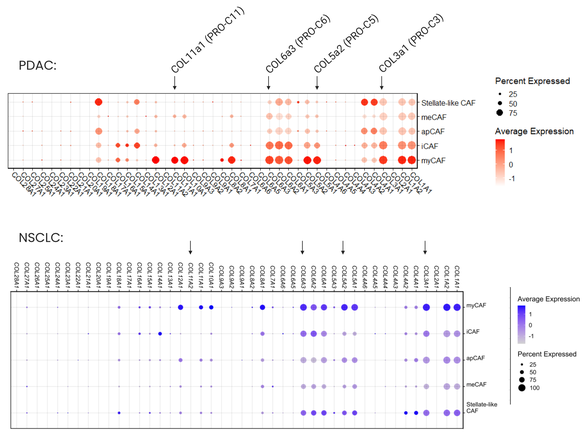
Clinical variables (PDAC)	Study population (n = 810)
Age, (years), Median (min, max)	66 (37-89)
Gender, n (% Female)	377 (47%)
BMI, Median (min, max)	23 (14-39)
Metastatic sites, ≥1 site, n (%)	434 (54%)
Stage 1+2	138 (17%)
Stage 3	237 (29%)
Stage 4	431 (53%)
Diabetes, Yes	198 (24%
Tobacco, Ever	484 (60%)
Alcohol, >Danish recommendation	179 (22%)
CA19-9, >median (>506 U/mL)	387 (48%)
Performance status, ≥2, n (%)	94 (12%)

CONCLUSION

Profiling collagen expression in fibroblast from PDAC and NSCLC reveals that type V collagen and type XI collagen are found in myCAF. Biomarkers of these collagens can be measured in serum from cancer patients and are prognostic for poor overall survival. Thus, these data suggest that cancer associated myo-fibroblast (myCAF) activity can be assessed non-invasively by specific collagen pro-peptide biomarkers.

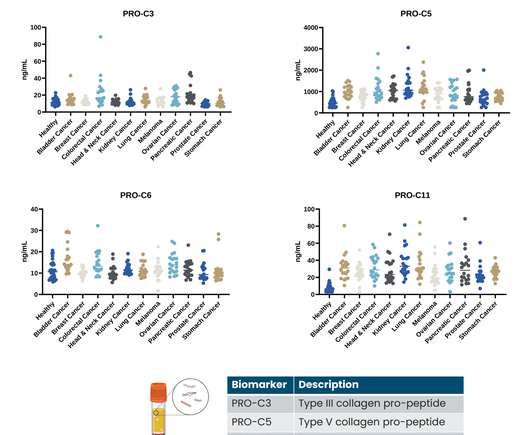
RESULTS

Collagen expression in CAF subtypes (scRNAseq data)



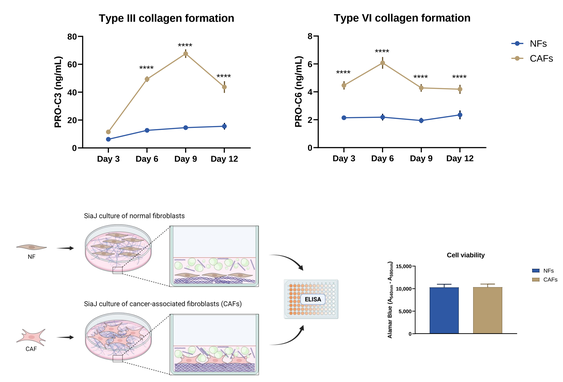
Thorlacius-Ussing et al., J Pathol 2023

Collagen pro-peptide profile in various indications, cohort 1 (serum)



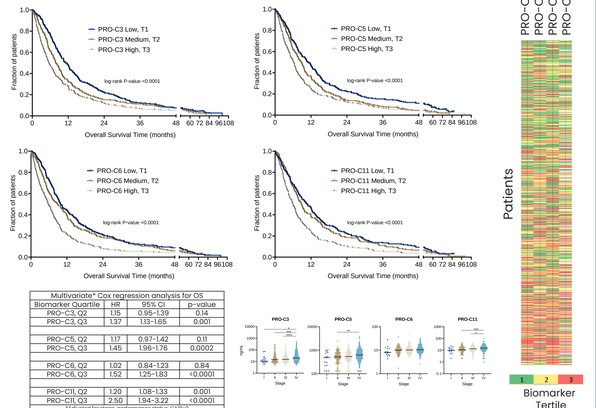
Biomarker	Description
PRO-C3	Type III collagen pro-peptide
PRO-C5	Type V collagen pro-peptide
PRO-C6	Type VI collagen, endothrophin
PRO-C11	Type XI collagen, propeptide

PRO-C3 and PRO-C6 expression in pancreas CAFs (in vitro)



Nissen et al., Cancers 2022

Prognostic value in pancreas cancer BIOPAC cohort (n=810)



Multivariate* Cox regression analysis for OS			
Biomarker Quartile	HR	95% CI	p-value
PRO-C3, Q2	1.15	0.95-1.39	0.14
PRO-C3, Q3	1.37	1.13-1.65	0.001
PRO-C5, Q2	1.17	0.97-1.42	0.11
PRO-C5, Q3	1.45	1.96-1.76	0.0002
PRO-C6, Q2	1.02	0.84-1.23	0.84
PRO-C6, Q3	1.52	1.25-1.83	<0.0001
PRO-C11, Q2	1.20	1.08-133	0.001
PRO-C11, Q3	2.50	1.94-3.22	<0.0001

*Adjusted for stage, performance status, CA19-9

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