The activity of fibroblast activation protein (FAP) is reflected by a specific fragment of type III collagen that can be serologically assessed and serve as a non-invasive biomarker

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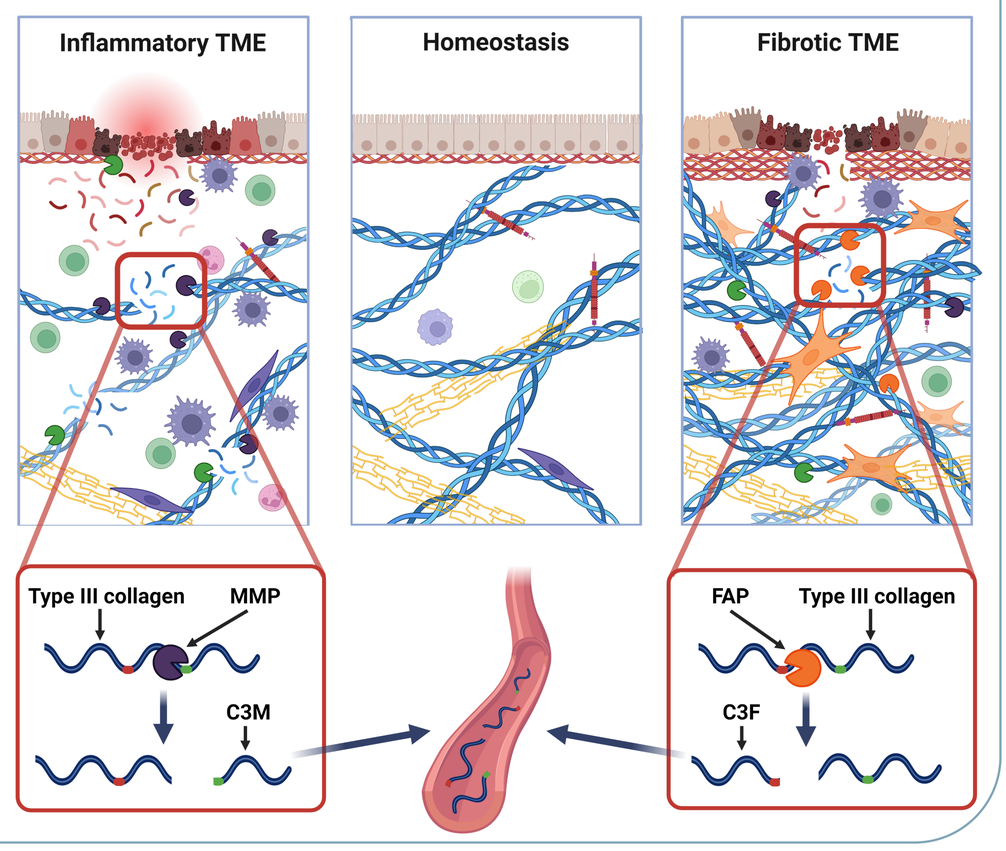
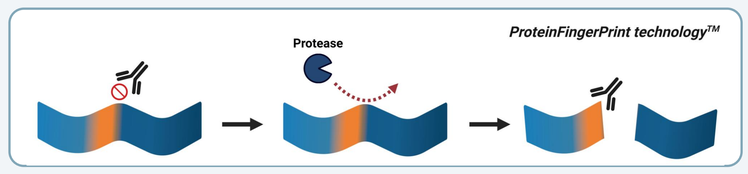
Background

- Non-small cell lung cancers (NSCLC) are associated with both inflammatory and fibrotic tumor microenvironments (TME).
- Inflammation is associated with an influx of macrophages, which are releasing matrix metalloproteinases (MMPs).
- MMPs are cleaving extracellular matrix proteins including type III collagen, which results in release of the C3M fragment into circulation.
- Tumor fibrosis is associated with accumulation of fibroblasts and high expression of Fibroblast activation protein (FAP), which is low or absent in benign tissue.
- FAP has unique proteolytic activity and have been shown to cleave various ECM proteins including collagens.
- We hypothesized that FAP-cleaved type III collagen would result in release of a specific fragment (C3F) into circulation, that could reflect FAP-activity in the TME.



Methods

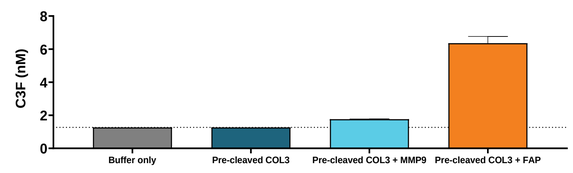
- To quantify FAP activity, we use *ProteinFingerPrint technology™* to develop a neo-epitope specific CLIA targeting C3F, a fragment specifically generated from FAP-mediated cleavage of type III collagen.



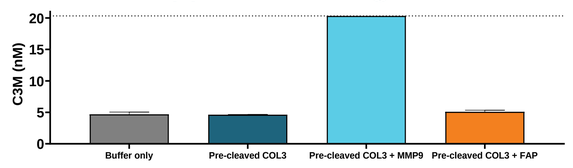
- Specificity of the biomarker towards FAP activity was compared by cleaving MMP13 pre-cleaved type III collagen with FAP or MMP9 followed by measurements of C3F and C3M.
- C3F and C3M were measured in serum from 65 patients with NSCLC and the correlation between the two biomarkers was investigated using Spearman's correlation.
- C3F was also measured in a cohort of 32 patients with NSCLC and 31 healthy individuals and the difference in C3F levels between the two groups was evaluated using Mann-Whitney test and AUROC.

C3F reflects FAP activity

The C3F fragment could only be detected with FAP-cleaved type III collagen and not with full length or MMP9-cleaved type III collagen.

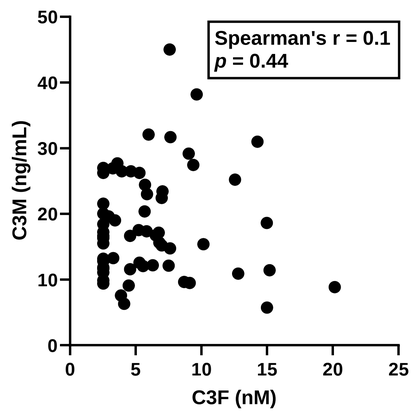


The C3M fragment could only be detected with MMP9-cleaved type III collagen and not with full length or FAP-cleaved type III collagen.



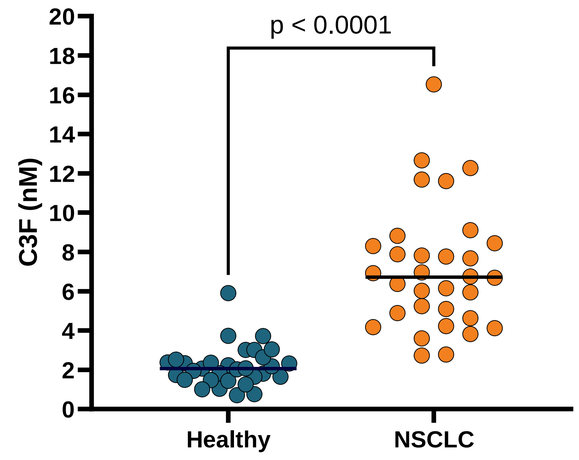
No correlation between C3F and C3M was observed

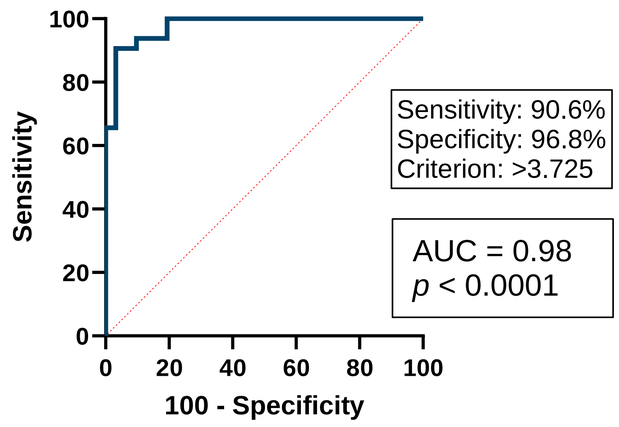
Serum levels of C3M and C3F in patients with NSCLC did not correlate, thus endorsing the claim that the two cleavage-fragments reflect different biological processes.



C3F showed diagnostic potential for patients with NSCLC

C3F was significantly elevated in serum from patients with NSCLC compared to healthy controls (*p < 0.0001) and showed diagnostic accuracy of 98%.*





Conclusion

FAP activity can be assessed by targeting a FAP-cleaved fragment of type III collagen, C3F and function as a non-invasive biomarker for patients with NSCLC.
By showing that this fragment differs from the MMP-generated fragment C3M, this study supports the notion that different biological processes are reflected in the proteolytic degradation of the ECM and can be reflected by specific circulating protein fragments.

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