From Target to Therapy: FAP Inhibitors and Their Potential in Cancer Theranostics

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Fibroblast activation protein alpha (FAP) is a dimeric serine protease with a highly selective expression pattern, particularly found on cancer-associated fibroblasts (CAFs) in the tumor microenvironment. Beyond its role in extracellular matrix degradation, FAP's restricted presence on activated fibroblasts and, in some cases, on tumor cells, makes it an attractive target for tumor imaging and therapeutic interventions. Our previous review with Dr. Bauvois explored various strategies for FAP-targeted cancer treatments. [1]

To facilitate the development of new FAP-targeting inhibitors (FAPIs), we optimized the recombinant human FAP (rhFAP) production in HEK293F cells, achieving significant improvements in yield and specific activity. This enabled us to perform a pilot cryo-EM study, resulting in a structure that shows promise for elucidating FAP-FAPI interactions at high resolution. Additionally, rhFAP is used to screen FAPI, including investigational PET radiotracers, via traditional IC50 assays. While these assays are fast and cost-effective, they are limited in evaluating tight-binding inhibitors and assessing kinetic behaviors.

To address these limitations, we developed comprehensive kinetic methods to characterize FAP-FAPI interactions, allowing for the selection of compounds with favorable binding kinetics. While my colleague Anke de Groot uses grating-coupled interferometry for binding studies, I focus on enzymatic Jump-Dilution assays and progress curves to determine k_{off}, aiming to predict the *in vivo* FAPI residence time. Determining additional key kinetic parameters such as k_{on} and the K_i will give a complete kinetic profile of these inhibitors. This assay is being further refined for implementation in the screening of FAPIs.

In collaboration with Nicolo Filippi, we are conjugating the FAPI 'UAMC-1110' to gold nanoparticles (AuNPs). [2] These nanoparticle-conjugated FAPI will be used for application in an *ex vivo* lateral flow assay for the detection of FAP in urine of bladder cancer patients. As an alternative for the FAPI, we are preparing to immunize llamas to raise single-domain antibodies against FAP.

In summary, our research advances the field of FAPI development by providing thorough *in vitro* FAPI characterization methods. Our efforts pave the way for more effective FAPI theranostics, to significantly impact tumor progression. On another note, we are pioneering an *ex vivo* FAP-targeting diagnostic application using AuNP-conjugated FAPIs for bladder cancer patient monitoring.

References

1. E. Verhulst, D. Garnier; Cancers, **14(3)**, 624 (2022).
2. K. Jansen, L. Heirbaut, J. Med. Chem., **57(7)**, 3053-3074 (2014).