DNA-encoded affinity maturation libraries enable the discovery of low picomolar Fibroblast Activation Protein inhibitors suitable for radioligand therapy of solid tumors

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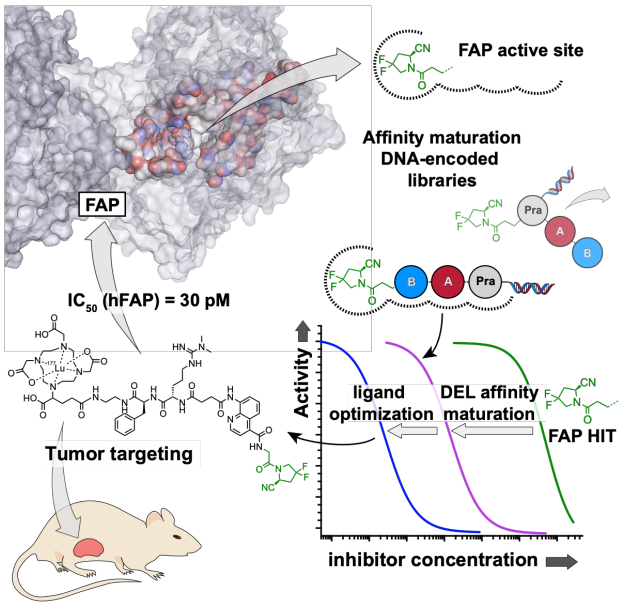
The discovery of potent ligands highly specific to accessible cancer-associated antigens is essential for the development of small molecule therapeutics with broad therapeutic index.[1] DNA-encoded chemical libraries (DELs) can be used to discover novel binders and to improve binding affinity of existing small organic ligands by affinity maturation procedures.[2,3] OncoFAP is a 680 pM inhibitor of the stromal pan-tumoral antigen Fibroblast Activation Protein (FAP).[4] OncoFAP displays best-in-class tumour targeting performance in patients, although its relatively short half-life limits the applicability as radioligand therapeutic against solid malignancies. We designed three focused affinity maturation DELs, containing 50,730 compounds bearing two sets of building blocks coupled to a propargylglycine central scaffold. Library selections against FAP from multiple species enabled the discovery of novel FAP inhibitors with potencies in the low picomolar range. ^{177}Lu-DOTAGA conjugates of the most potent novel ligands (named "**OncoFAP-11**" and **"BiOncoFAP-11"**) localized to tumours implanted in mice, with excellent tumor-to-blood and tumor-to-kidney ratios at late time-points after systemic administration. The high affinity FAP ligands described here enable the efficient delivery of therapeutic radionuclides to tumours with long residence time and with low uptake in normal tissues. The results presented here show that DEL-based affinity maturation procedures can lead to the improvement of the targeting performance of FAP binders, without compromising their tumour selectivity.



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[2] Neri and Lerner, *Annu. Rev. Biochem.*, **2018**, 87, 479–502.
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