Discovery of novel macrocyclic peptide radioligands for tumor therapy by mRNA display

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Introduction

Radioligand therapeutics (RLT) aim to selectively deliver radioisotopes to cancer tissues to eradicate tumor cells while limiting the damage to surrounding tissues. The therapeutic potential of RLT strongly depends on their biostability and tissue residence time after injection. Macrocyclic peptides are well suited as tumor-targeting ligands for RLT because their size and molecular characteristics confer optimal pharmacologic properties that can be fine-tuned during the development process: 1) high-affinity, 2) small in size improving tumor penetration, 3) short plasma half-life reducing off-target toxicity, 4) long tumor retention for optimal delivery of radiation dose, 5) no immunogenicity, 6) synthetic production facilitating modifications and shortening development timelines and costs[1,2].

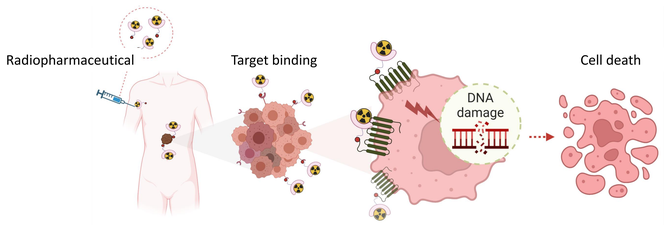

Figure 1. Radiopharmaceuticals selectively guide therapeutic radionuclide payloads to tumor cells for targeted therapy (Figure adapted from Desai *et al.*, 2022 by using BioRender).

mRNA display is a powerful platform for macrocyclic peptide drug discovery and has the potential to produce tumor-targeting macrocyclic peptides for RLT. mRNA display is an *in vitro* selection method that identifies high affinity macrocyclic peptide ligands to a target by enriching a highly diverse library (>10^{13} members)[4]. Here, we describe the use of mRNA display to discover macrocyclic peptides that bind to the extracellular domain of a tumor target. These peptides will be developed into radioligands and assessed for their ability to deplete solid tumors.

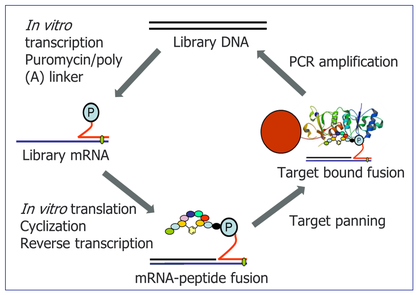

Figure 2. mRNA display method overview (Figure adapted from Ma and Hartman, 2012). An mRNA library is generated by *in vitro* transcription from library DNA and crosslinked to a puromycin/poly(A) linker. This mRNA library is converted to an mRNA-macrocyclic peptide fusion library by *in vitro* translation followed by cyclization and reverse transcription. During *in vitro* translation, the puromycin linker covalently links the peptides to the mRNA sequences that encode them. This enables amplification and detection of the peptides that bind the target immobilized on magnetic beads during the target panning step. The sequences of the fusions that bind the target are amplified by PCR and are the library input for the next round of selection. The selection is repeated until target binding enrichment of the library is observed.

mRNA display selection produced library enriched for target binding peptides

Binding enrichment of the peptide-mRNA fusion library for the recombinant target protein was observed after four rounds of selection with no further enrichment in round five. Peptide sequences present in each selection round were determined by Illumina sequencing. Clusters of related sequences in the enriched library were determined by hierarchical clustering of the top 1000 sequences with highest counts in round 5. 43 clusters were identified using a Hamming distance of 8.

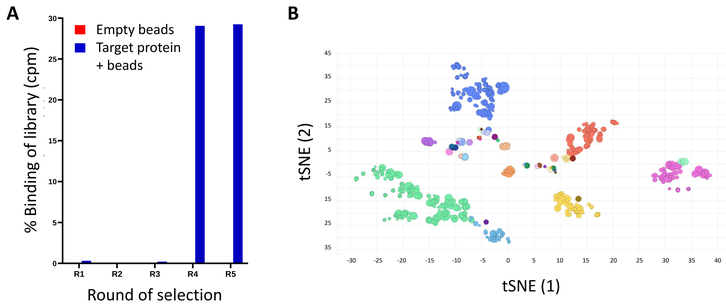

Figure 3. (A) Binding enrichment of library after each round selection. Significant enrichment over empty magnetic bead background beads was observed in R4 and R5. (B) tSNE (Stochastic Neighbor Embedding) projection of hierarchical clustering of top 1000 sequences in R5. Data point color corresponds to sequence cluster with Hamming cut-off = 8 and data point size is scaled by Log10 (sequencing counts).

Target binding screen of enriched library identifies peptide sequences for synthesis and hit confirmation

To select sequences for peptide synthesis and hit confirmation, individual macrocyclic peptides from each sequence cluster were produced by *in vitro* translation and screened for high affinity target binding. Most of the sequences tested show binding to the target protein and only a few show binding to a closely related protein from the same protein family.

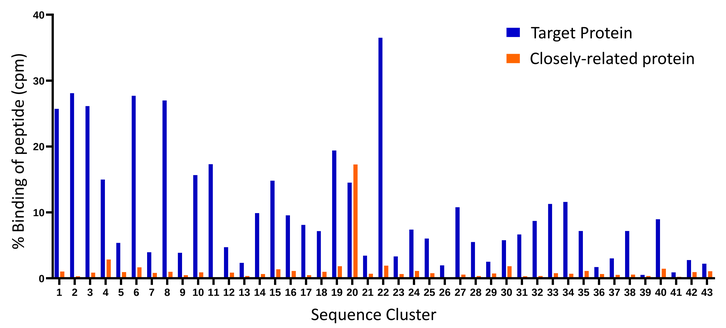

Figure 4. Percent binding of representative peptides from each sequence cluster to target and closely related target protein. Individual peptides were produced by *in vitro* translation and incorporated ^{35}S for detection of binding.

Binding affinity measurements confirm identification of high affinity macrocyclic peptide ligands for target

A subset of peptides was selected based on target binding screen results for chemical synthesis (purity >90%) and hit confirmation. Binding affinity to the target protein and a closely related protein was determined by surface plasmon resonance (SPR). 90% of analyzed candidates show high-affinity binding to the target with 45% showing K_D between 100-500 nM and 45% binding with K_D < 100 nM. Most analyzed candidates showed relatively weak or no binding to a closely related protein or irrelevant protein.

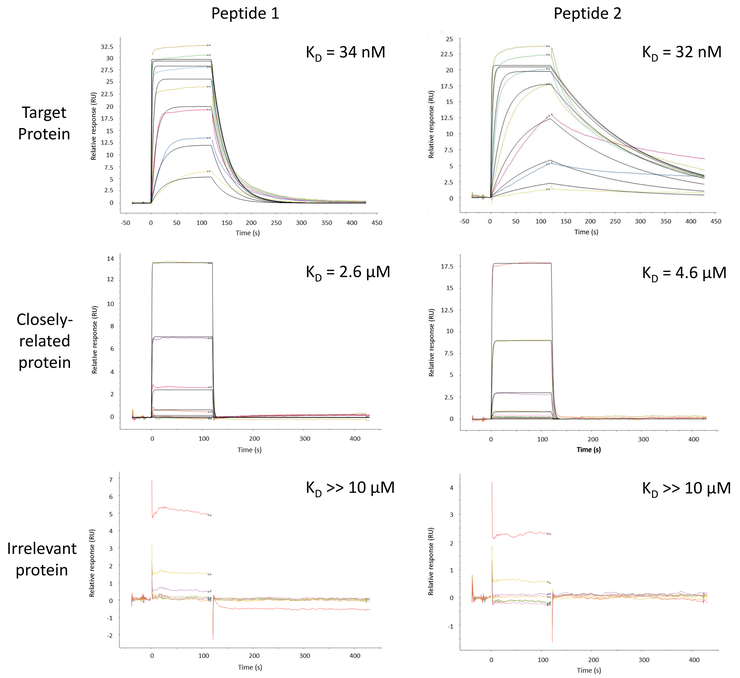

Figure 5. Exemplary SPR data of synthesized peptide hit candidates. Biotinylated target protein was immobilized on streptavidin biosensor chips and binding of purified peptides was tested at different concentrations up to 10 µM. Reported K_D values are an average of two experiments.

Conclusions & Outlook

By using mRNA display, we discovered novel macrocyclic peptides with high-affinity binding properties against a selected target in a short-time frame of 9 months.

A set of macrocyclic peptides selected based on SPR and kinetic solubility data will be further characterized and optimized for cellular binding, stability, *in vitro* anti-tumor efficacy, and *in vivo* biodistribution.

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References

1. Lepareur N, Ramée B, Mougin-Degraef M, Bourgeois M. Clinical Advances and Perspectives in Targeted Radionuclide Therapy. Pharmaceutics. 2023 Jun 14;15(6):1733. doi: 10.3390/pharmaceutics15061733. PMID: 37376181; PMCID: PMC10303056.
2. Zhang T, Lei H, Chen X, Dou Z, Yu B, Su W, Wang W, Jin X, Katsube T, Wang B, Zhang H, Li Q, Di C. Carrier systems of radiopharmaceuticals and the application in cancer therapy. Cell Death Discov. 2024 Jan 9;10(1):16. doi: 10.1038/s41420-023-01778-3. PMID: 38195680; PMCID: PMC10776600.
3. Desai P, Rimal R, Sahnoun SEM, Mottaghy FM, Möller M, Morgenroth A, Singh S. Radiolabeled Nanocarriers as Theranostics-Advancement from Peptides to Nanocarriers. Small. 2022 Jun;18(25):e2200673. doi: 10.1002/smll.202200673. Epub 2022 May 8. PMID: 35527333.
4. Newton MS, Cabezas Y, Seelig B. Advantages of mRNA display. ACS Synth. Biol. 2020 Feb 21; 9(2):181-90. doi: 10.1021/acssynbio.9b00419. PMID: 31891492
5. Ma Z, Hartman MC. In vitro selection of unnatural cyclic peptide libraries via mRNA display. Methods Mol Biol. 2012;805:367-90. doi: 10.1007/978-1-61779-379-0_21. PMID: 22094817.

Acknowledgements

The authors thank Manuel Sturzbecher-Hoehne[1], Dennis Mewis[1] and Liam Rayman[1] for their contributions in the discussion of the data. The authors thank Ellen Wallace[2], Ru-Ju Chian[2], Junru Wang[2], Terry Webb[2], Charlie Bu[2], Adam Hold[2], and Phil Pierce[2] for producing and characterizing the proteins used in this work. The authors also thank Kushi Aggarwal[2] for her contributions to SPR data acquisition.