Searching for peptide inhibitors of TNFR2/TNFα complex formation using a virtual "phage display"

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INTRODUCTION

- New hope for cancer treatment comes from immunotherapy, which is based on blocking immune checkpoints (ICPs). ICPs are proteins (receptors and their ligands) found on the surface of immune cells. To maintain homeostasis, the body forms receptor-ligand interactions that can both stimulate and inhibit the immune response.

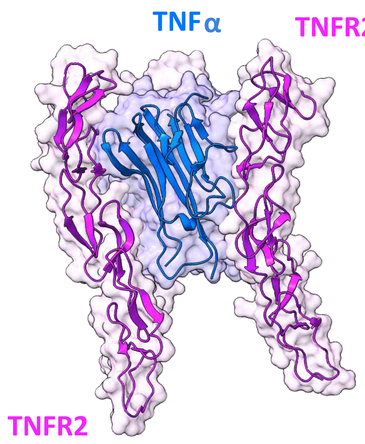

Fig. 1. *Crystal structure of TNFR2/TNFα complex [PDB: 3alq]*

- The receptor for tumor necrosis factor 2 (TNFR2) and its ligand, tumor necrosis factor (TNFα), constitute a stimulatory immune checkpoint.
- TNFR2 is mainly expressed on malignant cells and T-regulatory cells (Tregs). Its interaction with TNFα leads to the activation and proliferative expansion of Tregs, which subsequently suppress the immune response. This suppression interferes with the activation of cytotoxic T lymphocytes (Tc), whose function is the elimination of malignant cells.
- Moreover, ligation of TNFα on TNFR2 expressed on tumor cells initiates signaling pathways that promote tumor cell survival, proliferation, and metastasis. Consequently, the TNFR2/TNFα interaction contributes to tumor immuneevasion and development (Fig. 2A).

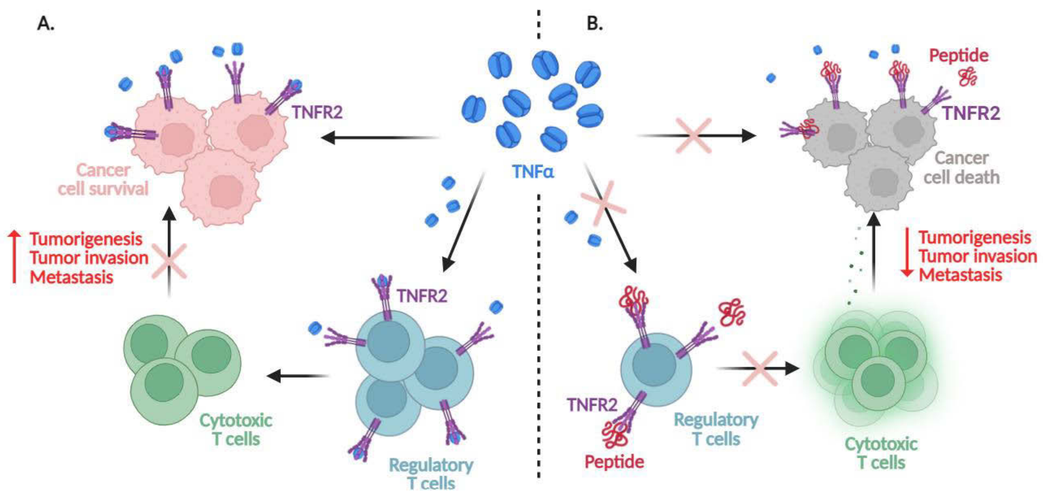

Fig. 2. A) *Tumorigenesis from TNFR2/TNFα complex formation;* ***B)*** *Inhibitor application obstructs TNFR2/TNFα complex formation and promotes tumor regression*

- Blockade of the TNFR2/TNFα interaction with peptide inhibitors can inhibit the activation of Tregs. Furthermore, the expression level of TNFR2 on the cell surface is reduced by the TNFR2 antagonist. As a result, the quantity and functionality of Tc are enhanced, leading to a more robust antitumor immune response (Fig. 2B).

RESULTS AND DISCUSSION

- The main objective of this research is to identify peptide inhibitors of TNFR2/TNFα complex formation. To achieve this, we utilized QSAR - PeptAIm software, which employs machine learning (ML) and artificial intelligence (AI) methods. The software conducted a virtual 'phage display' experiment, generating a peptide-ligands candidate referred to as Pep(1) (Fig. 3).

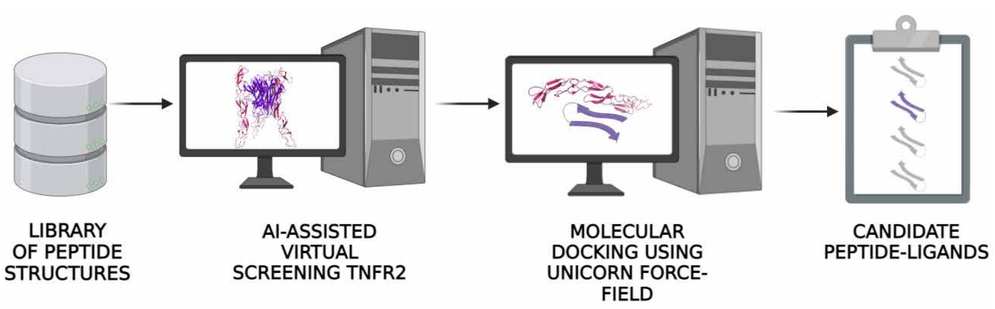

Fig. 3. *Scheme of virtual screening PeptAIm*

- Peptide Pep(1) was synthesized using solid-phase peptide synthesis (SPPS) with Fmoc/tBu chemistry and subsequently subjected to a biotinylation reaction. However, Pep(1) has not been obtained in pure form. Consequently, Pep(1) was modified to yield the Pep(2) - Pep(6) peptides.

Tab. 1. *Amino acid sequences of peptides selected for further research*

Peptide name	Amino acid sequence
Pep(1)	Biotin-^{1}SWNYNLDMGTWERS^{14}-NH_2
Pep(2)	Biotin -^{1}SWNYNLDSGTWERS^{14}-NH_2
Pep(3)	Biotin -^{1}SYNYNLDSGTYERS^{14}-NH_2
Pep(4)	Biotin -^{1}SYNYNLDSGTYRRS^{14}-NH_2
Pep(5)	Biotin -^{1}SYNYNLDSGTYRES^{14}-NH_2
Pep(6)	Biotin -^{1}SYNYNLDSGTYEES^{14}-NH_2

- Pep(2) - Pep(6) peptides were modified at positions 2, 8, and 11 due to the high propensity of Met and Trp for oxidation.

  In the Pep(4) - Pep(6) compounds, the peptide charge was also altered at positions 12 and 13. These changes occurred due to the substitution of acidic residues with basic ones in Pep(3) and Pep(4), and basic residues with acidic ones in Pep(5) and Pep(6).
- Peptides were synthesized using SPPS, subjected to a biotinylation reaction, and then purified and analyzed using RP-HPLC and MS techniques.

- The ability of Pep(2) - Pep(6) peptides to interact with the TNFR2 protein was assessed using an indirect enzyme-linked immunosorbent assay (Fig. 4).
- The biotinylated peptides (40µg/ml) were pre-coated on a streptavidin plate. In the next step, TNFR2-Fc was added at various concentrations. The addition of a secondary anti-human IgG antibody conjugated with HRP (horseradish peroxidase) enabled the detection of the Fc region in TNFR2. In the final step, TMB (3,3′,5,5′-tetramethylbenzidine) substrate was added to catalyze the enzymatic color reaction.

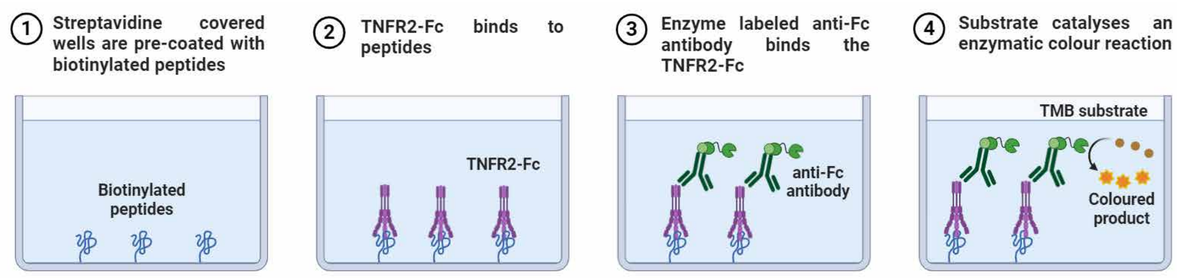

Fig. 4. *Scheme of indirect ELISA*

- The titration assay demonstrated that TNFR2 binds to all tested peptides in a concentration-dependent manner (Fig. 5). The strongest binding was observed with Pep(4), while a slightly weaker interaction was noted for Pep(5).

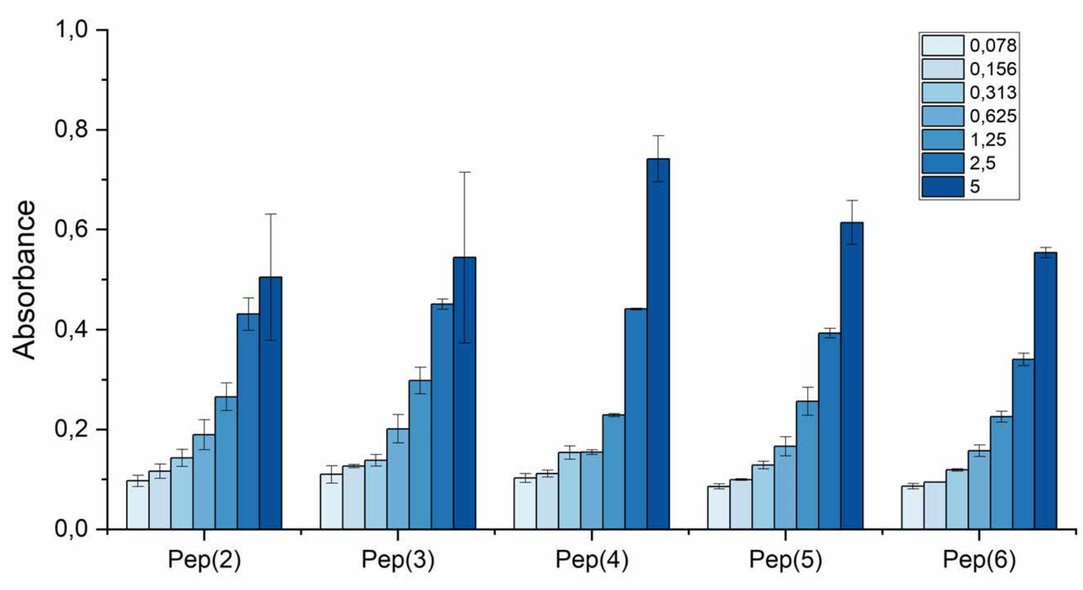

Fig. 5. *Binding of Pep(2) - Pep(6) peptides analysed using indirect ELISA. Data are depicted as mean with SD (Mean +/- SD).*

CONCLUSION

- The ELISA assay demonstrated that Pep(4) exhibited the strongest binding affinity for the TNFR2 receptor. These findings suggest that basic amino acid residues may enhance the interaction with TNFR2.
- The next step is to assess the ability of these peptides to inhibit the formation of the TNFR2/TNFα complex.
- The innovation of this research lies in the use of a specialized algorithm based on ML and AI, which reduces the costs and computational power required to develop receptor-binding sequences.

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