Post-Antibody Drugs: Generation of a novel class of drug modalities based on molecular-targeting helix-loop-helix (HLH) peptides

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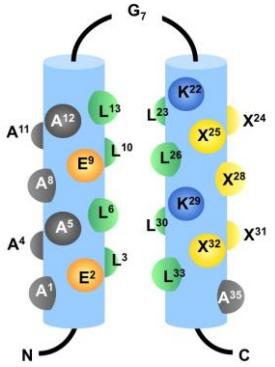
Antibodies are indisputably the most successful reagents in molecular-targeting therapy. However, the use of antibodies has been limited due to the biophysical properties and the cost to manufacture. To enable new applications where antibodies show some limitations, we have developed an alternative-binding molecule with non-immunoglobulin domain. The molecule is a helix-loop-helix (HLH) peptide, which is stable against enzyme degradations in vivo and is too small to show immunogenicity. Here, we introduce our HLH molecular-targeting peptides that show antibody-like functions, high affinity and high specificity for the targeted proteins.

Design of helix-loop-helix

- A helix-loop-helix is 35 amino acid residues
- *N*-terminal α-helix, *C*-terminal α-helix, Glycine linker
- 8 Leucines on the inside faces make a hydrophobic core to stabilize the structure.
- Glutamic acids and lysines on the side faces make a intrachain salt bridge to stabilize the structure and disturb the oligomerization.
- Outside amino acids (**X**) have no contribution for the structure stability, so then, they can be randomized to give a library of helix-loop-helix peptides.



Since the HLH peptide folds by virtue of hydrophobic and electrostatic interactions between the amino acid residues positioned inside the molecule, the outside solvent-exposed residues are possible to be mutated with a variety of amino acids to give a combinatorial library of the HLH peptides. Based on our technology of phage-displayed libraries for antibodies, we constructed a phage-displayed library of the HLH peptides. The library was screened against G-CSF receptor to give a binding peptide, which was cyclized by a thioether linkage between the N- and C-termini. The cyclic peptide showed a strong binding affinity (Kd of 4 nM) to the receptor and a long half-life (>2 weeks) in mouse sera, proving an enzyme-resistant property[1]. Immunization of the HLH peptide to mice showed no induction of the antibody production (non-immunogenic). We have applied our HLH peptide libraries for CTLA4[2], VEGF[3,4], kinases[5], HSA[6] to obtain their molecular-targeting HLH peptides. In addition, we used the HLH peptide as a scaffold for generating cell permeable targeting peptides through bi-functional grafting: epitope grafting to provide binding activity and arginine grafting to endow cell-permeability[7]. The HLH peptides provide insights into *de novo* peptide-based drug discovery and then would be a new therapeutic modality.

References

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