

論文 / 著書情報
Article / Book Information

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種別(和文)	論文要旨
Type(English)	Summary

論文要旨

THESIS SUMMARY

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要旨 (英文 800 語程度)

Thesis Summary (approx.800 English Words)

This thesis is entitled, “Development of target-binding small proteins using antibody-guided design and screening” and is composed of four chapters.

In Chapter 1, “General introduction,” a background of the study and the goals of my research are presented. Target-binding small proteins such as fluctuation-regulated affinity proteins (FLAPs) can serve as low-cost alternatives to current monoclonal antibody (mAb) biopharmaceuticals. FLAPs were developed based on the finding that target-binding peptides exhibit increased binding affinity upon immobilization within a protein scaffold. In this study, I aimed to develop FLAPs using two antibody-guided design and screening methods.

Chapter 2, “Identification of a HER2-binding FLAP through computational screening,” builds on a previous design strategy to create FLAPs made up of a structurally immobilized peptide derived from the complementarity-determining region (CDR) loops of mAbs and a stable protein scaffold. Because the paratopes of mAbs are generally composed of multiple CDRs, it was hypothesized that FLAPs with multiple binding peptides may present enhanced target-binding capability compared to those with only one. In this chapter, the development of a strategy to create FLAPs bearing dual CDR peptides (D-FLAPs) derived from the anti-human epidermal growth factor receptor 2 (HER2) mAb biopharmaceutical, trastuzumab, is described. First, CDR-derived peptides containing target-binding residues were computationally selected. These peptides were then grafted into two adjacent loops of the fibronectin type III domain (FN3) scaffold protein, yielding a small library of 80 D-FLAP candidates. Following computational screening based on the conformational similarity of the target-binding residues to those on the parental mAb, two candidates, FN3.38 and FN3.58, were selected. After bacterial expression and purification of the two proteins, enzyme-linked immunosorbent assay (ELISA) results revealed that FN3.58, hereby referred to as D-FLAP, which possesses the peptides HYTPP and GDGFYA from CDRs-L3 and H3 of the parental mAb, respectively, bound HER2 with a dissociation constant of 58 nM. Furthermore, the results of ELISA also revealed that both the light- and heavy-chain CDR peptides contribute to binding, as the lack of either one resulted in decreased binding affinity. Finally, to further investigate the binding of D-FLAP to HER2, docking simulations were performed. The proximity of the CDR-derived peptides to the target protein in the top three docks support the finding that the CDR-derived peptides mediate binding. Additionally, binding energy calculations using the top 3 docks revealed that although the heavy-chain CDR peptide appears to contribute more directly to binding, the light-chain CDR peptide

may play a supporting role, ultimately resulting in increased binding affinity.

In Chapter 3, “Identification of a CD25-binding FLAP through antibody-guided mammalian display screening,” the development of a novel peptide screening system is described. In the system, a two-pronged approach is taken to identify FLAPs using a mAb to “guide” the process. First, antibody-guided design allows the construction of peptide libraries that are relatively smaller in size but sufficient for identifying target binders in a single selection round. Second, antibody-guided screening in which both the target antigen and peptide library are displayed on the surface of mammalian cells, and in which the fluorescent label-based detection of peptide binding to the antigen is facilitated by the same mAb used to design the library, allows the identification of high affinity binders. To demonstrate its utility, the system is used to screen a constrained peptide library designed based on the anti-interleukin-2 receptor alpha chain (CD25) mAb. Three out of the top five candidate peptides were found to bind CD25 specifically with binding affinities of approximately 30 nM as measured by biolayer interferometry (BLI), without undergoing affinity maturation.

Chapter 4, “Conclusion and prospects,” summarizes the results of this study and provides issues to consider in order to further improve the method of FLAP development. Briefly, through my research, I aimed to efficiently develop FLAPs by employing two novel methods that use mAbs to guide both library design and screening. For the first method, I developed an anti-HER2 FLAP called D-FLAP by immobilizing two peptides derived from the anti-HER2 mAb trastuzumab within the FN3 scaffold and screening candidates based on their structural similarity to the parental trastuzumab CDR. For the second approach, I established a modified mammalian display system and used it to screen a library designed by immobilizing anti-CD25 mAb CDR-derived residues within a scaffold peptide to identify CD25-binding FLAPs. The two methods are applicable to any mAb, and can be used to efficiently identify new FLAPs that can serve as alternatives to mAbs. Although the binding affinities of the created FLAPs were not as high as those of their parental mAbs, their moderate binding affinities may yield better safety profiles and lower toxicity. Moreover, they have the additional advantage of chemical synthesizability, which is cheaper than current production methods used to manufacture mAbs. The results of my research demonstrate that these two methods are promising and versatile tools for the development of FLAPs.

備考：論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

Note: Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1 copy of 800 Words (English).

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