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Article / Book Information

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Title(English)	Development of HER2-targeting small proteins by immobilization of binding-peptides in scaffold proteins
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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Life Science and Technology 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Engineering)
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

This thesis is titled "Development of HER2-targeting small proteins by immobilization of binding-peptides in scaffold proteins" and is composed of 6 chapters.

Chapter 1 "General Introduction" describes the research background and purpose of this study. Small proteins that chemically synthesizable and specifically bind to cancer cell markers are promising alternatives to antibody (Ab). These small proteins overcome some limitations of Ab such as high molecular weight, limited tissue penetration, and high production cost. Therefore, various types of Ab alternative small proteins have been extensively developed. Previous study revealed that a structural immobilization of the target-binding peptides by grafting to a particular site of a fluorescent protein scaffold increases their binding affinity. In this study, I aimed to establish a technology to create high-performance target-binding small proteins, fluctuation-regulated affinity proteins (FLAPs), by immobilizing target-binding peptides into small protein scaffolds.

Chapter 2 “Identification of small protein scaffolds and grafting acceptor sites” explains the selection of small protein scaffolds and identification of regions suitable for grafting peptides (GA sites) by MD simulation. I selected 13 human-derived protein scaffold candidates that had sizes amenable to chemical synthesis and a low number of disulfide bonds from protein database. As a result of evaluation by a unique method using the Root Mean Square Fluctuation (RMSF) values in MD simulation as an index, I selected 13 GA sites in 6 scaffolds for FLAP construction.

Chapter 3 “Development of FLAPs using HER2-binding peptide derived from anti-HER2 monoclonal Abs” describes the design and evaluation of AbP-FLAPs harboring Ab-derived HER2-binding peptides (AbPs) extracted from structures of Ab drugs that bind to breast cancer marker HER2. I designed 65 FLAP candidates by grafting five AbPs extracted by binding energy calculations of Abs, trastuzumab and pertuzumab, into the 13 GA sites identified in Chapter 2. Among them, I found three AbP-FLAPs that have trastuzumab-derived AbPs into fibronectin type III (FN3) scaffold protein bind to the same

epitope as trastuzumab (K_D = 270–350 nM).

Chapter 4 “Development of FLAPs by grafting HBPs” describes the design and evaluation of HBP-FLAPs harboring HER2-binding peptides (HBPs) identified by screening peptide libraries. I succeeded in creating an HBP-FLAP (K_D = 287 nM) by grafting an HBP isolated from a phage-displayed cyclic peptide library into the GA sites and affinity maturation. In addition, I found proteolytic resistance of HBP is greatly improved in HBP-FLAP.

Chapter 5 “Specific binding of FLAPs to HER2-overexpressing cells *in vitro* and *in vivo*” describes the applicability of FLAPs as diagnostic probes. For *in vitro* immunostaining, three AbP-FLAPs and one HBP-FLAP bound to HER2-overexpressing SK-BR-3 and N87 cells but hardly to control HeLa cells that express low or no HER2. For *in vivo* imaging, HBP-FLAP was able to delineate HER2-overexpressing tumors with a half-life of 6 h after intravenous injection to the tumor-bearing mice. These results indicate that FLAPs specifically bind to the target and are applicable to both *in vitro* and *in vivo*.

Chapter 6 “Conclusion and prospect” summarizes the results of this study and describes future prospects. In this study, I have demonstrated a successful creation of FLAPs by grafting structurally immobilized AbPs or HBPs into small protein scaffolds. A strategy for creation of AbP-FLAPs is applicable for other monoclonal Abs and other functional proteins to develop their small protein alternatives. While a strategy for creating HBP-FLAPs is useful for not only developing clinically applicable small proteins, but also increasing proteolytic resistance of grafted target-binding peptides. Although the binding affinity of created FLAPs is moderate, this may have the advantage of reducing toxicity due to on-target off-tumor binding. Overall, I hope that the efficient and practical method of FLAP development can be applied to many therapeutic targets, not only cancer but also other diseases where antibodies and recombinant proteins are currently used. Therefore, this study may open new avenues for developing novel technology platforms in protein engineering and biopharmaceutical design.

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