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論文 / 著書情報
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種別(和文)	論文要旨
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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 ライフエンジニアリング	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	（ 工学 ）
学生氏名： Student's Name	Ning Lin		審査員主査： Chief Examiner	門之園 哲哉	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Epitope, the specific region of antigen recognized by an antibody, is inherently related to the functionality of antibody drugs. Epitope characterization represents a critical step during antibody drug development, providing valuable insights into comprehending the molecular mechanisms of antibodies and evaluating the therapeutic potential of antibody drug candidates. Epitope mapping can reveal binding information at residue-resolution through various techniques, such as X-ray crystallography, nuclear magnetic resonance, hydrogen/deuterium exchange, and cryo-electron microscopy. However, these techniques require high-quality samples for analysis, rendering them labor-intensive, time-consuming, and unsuitable for large-scale analysis. An alternative for epitope mapping is epitope binning, which categories a large number of antibodies into different bins based on epitope similarities. While epitope binning cannot provide precise binding residue information, such antibody classification remains valuable for the rational selection of the most promising candidates for further evaluation and development. Epitope binning utilizes pairwise competitive binding assays to determine whether query antibodies (qAbs) and reference antibodies (rAbs) target overlapping or different epitopes on the same antigen. This is accomplished through detecting competitive binding inhibition between qAb and rAb. The competitive binding strategy can be integrated with various techniques, including enzyme-linked immunosorbent assay, biolayer interferometry, surface plasmon resonance, and flow cytometry (FCM). However, currently available approaches require the individual production and even purification of antibodies, thus limiting their utility for large-scale evaluations. To address this issue, the purpose of my study is to develop a method capable of eliminating the need of individual antibody production and enabling the simultaneous evaluation of multiple antibodies.

A screening system, called monoclonal antibody-guided peptide identification and engineering (MAGPIE), has been developed for identifying antigen-binding peptides [*Sci Rep*, **11 (1)**, 22098 (2021)]. In MAGPIE, both the antigen and the peptide library are displayed on the surface of mammalian cells, and peptide selection is based on competitive binding between the displayed peptide and an exogenous antigen-binding antibody. Since the fundamental principle behind MAGPIE is consistent with that of epitope binning, it is expected that the epitope of the identified antigen-binding peptides should be similar to that of the gAb. In this study, I aim to extend the antigen/antibody co-display system as a platform for epitope similarity evaluation and epitope

binning.

As a proof of concept, the human epidermal growth factor receptor 2 (HER2) and the anti-HER2 monoclonal antibodies, pertuzumab and trastuzumab, were utilized as the model antigen and rAbs, respectively. The single-chain variable fragment from either the rAbs or HER2-non-binding antibodies is displayed on HER2-expressing cells as qAbs. FCM analysis of HER2/qAb co-display cells with a fluorescently labeled rAb revealed specific binding inhibition of the rAb by the surface-displayed qAb with the same epitope, as evidenced by the presence of an rAb-non-binding [rAb(-)] cell population in cells expressing a qAb targeting the same epitope as the rAb, rather than those with a different epitope or non-binding qAb. Furthermore, when two different rAbs were used simultaneously, corresponding rAb(-) cell populations could be observed. These findings demonstrate that epitope similarity between the qAb and rAb could be evaluated using this antigen/qAb co-display system, under the condition of either a single rAb or dual rAbs.

Moreover, I investigated several critical factors influencing the sensitivity of epitope similarity evaluation, including the concentrations of rAb, the relative abundance of antigen and qAb, as well as the affinity of qAb. To assess the effect of rAb concentrations, cells co-expressing antigen and qAb were incubated with varying concentrations of rAb, revealing that low concentrations (0.1-10 nM) are advantageous for the evaluation. Next, the effect of the relative levels between qAb and antigen was investigated from two aspects. Firstly, the expression levels of qAb and antigen in rAb(-) and rAb-binding [rAb(+)] populations within the same cell line were estimated, revealing higher qAb but lower antigen expression in the rAb(-) compared with the rAb(+) population. Secondly, using cell lines with the expression levels similar in qAb but different in HER2, I analyzed the percentage of rAb(-) population and found that higher sensitivity could be achieved using cells with a higher qAb/antigen ratio. For investigating the effect of qAb affinity, qAbs with varying affinities to HER2 but targeting the similar epitope were displayed on HER2-expressing cells and reacted with rAb. The evaluation sensitivity was less affected by qAb affinity at low rAb concentrations, while it declined with the reduction of qAb affinity at a high rAb concentration.

Finally, the antigen/qAb co-display system was integrated with next-generation sequencing (NGS) for epitope binning, which called Epitope Binning-seq. To validate the feasibility of this platform for parallel epitope binning, a model cell library displaying 14 different qAbs were reacted with each rAb, and the rAb(-) cells were isolated for NGS analysis. By analyzing the specific enrichment of each qAb in particular rAb(-) populations, qAbs sharing the similar epitope with the rAb were identified and categorized into respective epitope bins.

This study has provided a proof-of-concept for Epitope Binning-seq, a platform based on mammalian cell surface display and DNA sequencing. Epitope Binning-seq enables simultaneous analysis of multiple rAb-qAb pairs without the need for individual antibody production. It would be applicable across a broad range of antigens and antibodies, holding great promise for significantly facilitating antibody drug discovery.

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 1copy of 800 Words (English).
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