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Article / Book Information

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（博士課程）
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論 文 要 旨

THESIS SUMMARY

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

Although intracellular biomarkers can be imaged with fluorescent dye(s)-labeled antibodies, the use of such probes for the precise imaging of intracellular biomarkers in living cells remains challenging due to background noise from unbound probes. The Quenchbody (Q-body) is a newly developed fluorescent immunosensor with the potential to solve this issue. It is a site-specific fluorescent dye(s)-labeled antibody fragment that exhibits antigen-dependent fluorescence signal enhancement. Unlike conventional antibody probes, Q-bodies have the advantage of antigen-dependent signal generation. In this study, to investigate the applicability and feasibility of using Q-body technology in the imaging and dynamics monitoring of intracellular protein of interests (POIs) in living cells and intracellular antigen-specific live-cell sorting, the development of a conditionally active Fab-type Q-body probe derived from a monoclonal antibody (DO-1) with the ability to both targets and spatiotemporally visualize intracellular p53 in living cells with low background signal as described.

In Chapter 2, a mutant (C11) of WT_scFv DO-1 with improved secretion productivity was selected by constructing a consensus mutagenesis library and phage display bio-panning. The C11_scFv mutant showed an approximately 7-fold increment of signal in the monoclonal-secretion antibody ELISA assay. To understand the effect of mutation site introduction on the Q-body activity [e.g., limit of detection (LOD), maximum response, and EC_{50}], single-labeled WT_scFv and C11_scFv Q-bodies were prepared. The C11_scFv showed an increased sensitivity (LOD = 0.028 nM) in comparison to WT_scFv Q-body (LOD = 0.076 nM). Their EC_{50} and maximum response were not changed too much. A Q-body (C11_Fab -body) with a maximum response of 27-fold and LOD of 0.78 nM against only human p53 peptide was obtained by labeling the C11_Fab with two TAMRA dyes. And it was verified that the dye labeling showed minimal perturbation to the antigen-binding affinity. Besides the quenching mechanism of the dyes in the C11_Fab Q-body was investigated and suggested that both Trp residues and H-dimer formations contribute to the fluorescent quenching in this Q-body. And the fluorescent dye to protein (F/P) ratio and quantum yield of self-quenched and activated forms of Q-bodies were investigated. The results indicated that C11_Fab was efficiently labeled with TAMRA and the C11_Fab Q-body showed antigen-dependent quantum yield that was consistent with its fluorescence intensity changes.

In Chapter 3, after obtaining a high-performance Q-body, the one-step immunofluorescence assay was performed to investigate the ability to use the C11_Fab Q-body in the visualization of p53 in fixed cells, a

representative of traditional immunoprobe C11_scFv-TAMRA was used as a control. The results showed that the C11_Fab Q-body displays an antigen-dependent signal on fixed human cancer cells that either harbor WT p53 or mutant p53 and shows a higher S/B ratio than the traditional IF probe. Besides, this Q-body showed no fluorescent enhancement in mouse cells, indicating that the C11_Fab Q-body specifically recognizes human p53.

In Chapter 4, after confirming that the C11_Fab Q-body displays antigen-dependent signal turn-on in fixed cell image, next, its applicability to live-cell imaging was evaluated. The ability of visualization of both WT and mutant p53 in living cells was evaluated followed by delivery of the C11_Fab Q-body into the cytosol using electroporation. It showed fluorescence enhancement in the p53 expression cells irrespective of mutation types of p53 in comparison to p53-negative cells, indicating that Q-body shows antigen-dependent fluorescence enhancement in the complex intracellular environment of live cells.

In Chapter 5, the intracellular live-cell imaging assay was performed to further evaluate the stability and performance of the C11_Fab Q-body visualizing the p53 dynamics under the treatment of nutlin-3a (increase p53) and cisplatin (decrease p53). The fluorescent signals fluctuated p53 levels dependently. While in p53-negative cells, the signals were almost unchanged and kept at a low level. These data indicate that the Q-body is stable enough for long-term live-cell imaging and enables visualization of p53 dynamics in live cells. These pilot studies demonstrate that Q-body technology can be utilized to localize intracellular POIs in viable cells, but also allows visualization of the dynamic changes in intracellular targets in living cells.

In Chapter 6, to finally investigate the feasibility of applying this technology to intracellular antigen-specific live-cell sorting, a proof-of-concept experiment was performed using fluorescence-activated cell sorting (FACS). The ratio of p53-positive cells was enriched from 6.9% (before sorting) to 94% (after sorting) indicating that the application of Q-body technology in intracellular antigen-specific live-cell sorting is possible.

Taken together, this study provides the first evidence of the feasibility and applicability of Q-body probes for the live-cell imaging of intrinsically intracellular proteins and opens a novel avenue for research and diagnostic applications on intracellular target-based live-cell sorting.

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