論文の内容の要旨

Application of Immuno-PCR to sensitive detection of biomarker antigens
（バイオマーカー抗原高感度検出のためのイムノ PCR 法の応用）

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An immunoassay is a useful biochemical assay method that can specifically and sensitively measure target analytes in a sample solution. The assay is of critical importance in the fields of biological sciences, clinical diagnosis, and also environmental pollutant detection. Immunoassay is quite simple and less expensive in terms of quick operation, good sensitivity and specificity. Biomarker is a substance whose detection indicates a particular disease state, more specifically, a biomarker indicates a change in expression or state of a protein that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment. To date, many biomarkers are found for the specific diseases, and many of them are small molecules. Hence this study focuses on the detection of low molecular weight biomarkers with higher sensitivity than conventional.

In the first chapter of PhD thesis, I overviewed this and related area and made introduction to the following three topics, which are 1) application of phage-based immuno-PCR for the sensitive detection of small biomarker antigens by open sandwich immunoassay (OS-IA), 2) application of fusion protein-based immuno-PCR for the sensitive and practical detection of small molecules by OS-IA, and 3) application of SA-scFv fusion protein for the sensitive detection of avian influenza virus.

In Chapter 2, detection of two biomarkers by phage-based OS immuno-PCR and ELISA is described. Nowadays, most popular immunoassay is enzyme-linked immunosorbent assay (ELISA), and especially sandwich ELISA using two antibodies is preferred whenever possible. However, sandwich ELISA has a fundamental limitation that only polyvalent antigens with two antibody binding sites (epitopes) can be detected. To overcome this limitation, I employed open sandwich (OS) immunoassay that exploits antigen-dependent association of the two antibody variable region fragments V_H (heavy chain variable region) and V_L (light chain variable region). In this chapter, I attempted to apply this OS principle to the detection of two small biomarkers BGP peptide and estradiol, using the phage displaying V_H fragment and maltose-binding protein (MBP) fused with V_L (MBP-V_L) immobilized to the microplate. To perform OS ELISA, after phage displaying V_H on its surface is added with antigen, anti-M13 monoclonal antibody conjugate is added and substrate is added to detect antigen. In case of immuno-PCR, phage DNA is extracted by simple boiling, and the DNA is amplified by realtime PCR to quantitatively detect the DNA. I could show that the OS Immuno-PCR outperformed OS ELISA in the both detection in its detection limit and working range. Namely, phage-based OS-ELISA detects 100 pg/ml BGP-C7 and 10 ng/ml E2 antigen, respectively, whereas phage based OS Immuno-PCR detects 10 pg/ml BGP-C7 and 0.1 ng/ml E2.

In Chapter 3, to compare the results above with more popular protein-based approach, protein-based OS-ELISA and corresponding OS immuno-PCR were attempted. To this end, MBP-V_H for BGP peptide is prepared and coated onto the plate, and streptavidin-V_L fusion protein (SA-V_L) against this antigen is used for the detection. For ELISA, after adding this protein with antigen, biotin-HRP is used

for the detection, whereas for immuno-PCR, biotinylated DNA was used for the amplification and quantification by real-time PCR. The comparative results of OS-ELISA and Immuno-PCR showed improved detection sensitivity of OS immuno-PCR. The similarity and difference of phage-based and protein-based approaches are discussed based on the results.

In the Chapter 4 of this thesis, attempt to enhance the detection sensitivity of HA protein derived from avian influenza virus H5N1. The variable region gene for a specific antibody was cloned from hybridoma, assembled as a single chain Fv (scFv), and fused with SA to make a fusion protein. The SA-scFv fusion protein was produce in E. coli, and used for immuno-PCR with immobilized HA protein. The comparative study of ELISA and immuno-PCR showed improved sensitivity of immuno-PCR.

In the Chapter 5 of the thesis**,** conclusion and perspective are described. This would be the first trials of combining the merits of immuno-PCR and OS-IA, constructed with antibody fragments either displayed on phage or fused with proteins. This system will be applied to the sensitive detection of many small molecules including diagnostic markers and food contaminants. Also, the technology will be applied to the detection of important pathogenic viruses.