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DOCTORAL THESIS

Analysis of biomarkers of age-related diseases by total internal reflection fluorescence microscopy

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ABSTRACT

Total internal reflection fluorescence microscopy (TIRFM) has been widely applied for the study of biomolecules because of their ability to quantify biomolecules in a sample pretreatment and enrichment free manner, when compared with those costly, sample consuming and labor intensive conventional detection assay. This thesis presented the application of the TIRFM imaging system for the direct quantification and analysis of the biomarkers for the age-related diseases. Four research works on the quantification and study of biomarkers with the aid of TIRFM were herein described.

In the first detection scheme, an ultra-sensitive detection assay for direct quantification of protein biomarkers of Alzheimer's Disease (AD) was developed. In the assay, three protein biomarkers for the Alzheimer's disease were chosen as the target analytes, which were first captured by the magnetic nanoprobes and the secondary antibody. The magnetic immunocomplexes were labeled with a tailor-made indolium-based turn-on fluorophore, SIM, and the signal was then detected by TIRFM imaging system. The detection limit of $A\beta_{42}$, tau_{441} and p-tau_{181} were 23, 14 and 34 fM respectively. The detection assay was able to quantify the biomarkers in different types of biological fluids including cerebrospinal fluid, serum, saliva, and urine. With a minor modification, the detection assay was capable of detecting two biomarkers using TIRFM imaging system simultaneously.

To further improve the detection assay, another approach for the quantification of those three biomarkers were demonstrated. The secondary antibody was replaced by the aptamer and the signal is further amplified by amplification probes. With the addition of amplification probe and another tailor-made turn-on fluorophore, SPOH, the sensitivity of the detection is further improved by about 2.7- to 9.4-fold. The detection limit of $A\beta_{42}$,

tau_{441} and p-tau_{181} are 8.4, 4.3 and 3.6 fM respectively. The detection assay is able to quantify the biomarkers in both cerebrospinal fluid and serum with both TIRFM and conventional spectrofluorimeter.

Other than the protein biomarkers, the miRNA is also another type of biomarker which usually associated with cancer. A highly sensitive detection of miRNAs based on the ligation reaction on magnetic nanoparticles were developed. The detection assay was able to quantify the down-regulator hsa-mir-149 (mir-149) in serum. The magnetic nanoprobes for target miRNAs, mir-149, were prepared by conjugating the biotinylated DNA with the streptavidin modified iron oxide nanoparticles to remove the background matrix interference in serum. The locked nucleic acid modified molecular beacon (LNA/MB) of complementary sequence for mir-149 and reporting probe were first hybridized with target mir-149. The duplexes were then captured by the magnetic nanoprobes and amplified by poly-A and poly-T, followed by the ligation reaction. The magnetic hybrids were final labelled with intercalating fluorescence dye YOYO-1 and the signal was detected by the TIRFM-EMCCD imaging system. The assay demonstrated that efficient discrimination of single-base mismatch. A detection limit of 314 fM was achieved. The developed assay was capable of detecting the down-regulator mir-149 in serum with only 10 μL of serum. The applicability of quantifying circulating mir-149 in serum sample from both normal and cancer patient was also demonstrated.

Other than quantification, another application of the TIRFM imaging system is studying the effect of different compound on the growth of amyloid beta (one of the hallmark of AD). TIRFM was applied to monitor the growth of Aβ fibrils in the presence of Zn-containing compounds. We demonstrated that both disulfonimide-substituted phthalocyanine and Zn-based polymer exhibited inhibitory effect on the $A\beta_{1-40}$ peptide aggregation. Moreover, we

also demonstrated that inhibitory effect of the Zn-based polymers varied depending on both of the enantiomers and length of the helical diameter.

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