DOCTORAL THESIS

Direct quantification of cancer biomarkers by fluorescence microscopy

Ho, See Lok Ashley

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Direct Quantification of Cancer Biomarkers by Fluorescence Microscopy

HO See Lok Ashley

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Principal Supervisor: Dr. LI Hung Wing

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DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature: _____

Date: February 2015

ABSTRACT

As a high-resolution wide-field near-surface microscopy, total internal reflection fluorescence microscopy (TIRFM) has been widely applied for the study of biomolecules. Unlike those costly, sample consuming and time consuming traditional detection assays, the application of TIRFM enable the direct quantification of biomolecules in a sample pretreatment and enrichment free fashion. Taking advantages of the TIRFM imaging system, in this thesis we have applied the TIRFM imaging system to directly quantify the content of different cancer associated biomarkers. Four different detection approaches for direct cancer biomarkers quantification with the aid of TIRFM were herein presented respectively.

In Chapter 2, a direct quantification of nasopharyngeal carcinoma associated miRNAs was described. In the assay, five different miRNAs were chosen as the target analytes, which hybridized with the synthetic complementary LNA, probe in solution. The duplex was labeled with intercalating fluorescence dye YOYO-1 and the signal was then detected by the TIRFM-EMCCD imaging system. The LNA probe exhibited a high binding affinity towards the complementary target miRNAs and a limit of detection of 8 pM was achieved. Since the LOD is far below the reported concentration of miRNAs found in body fluids, this developed assay is of high potential to serve as a tool for non-invasive detection of miRNAs for early disease diagnosis.

In Chapter 3, an advanced single-molecule based assay for direct circulating miRNAs detection was developed. The assay was demonstrated to be capable of

differentiating the expression of a nasopharyngeal carcinoma (NPC) up-regulator hsa-mir-205 (mir-205) in serum collected from patients of different stages of NPC. To overcome the background matrix interference in serum, locked nucleic acid modified molecular beacon (LNA/MB) was applied as the detection probe to hybridize, capture and detect target mir-205 in serum matrix with enhanced sensitivity and specificity. A detection limit of 500 fM was achieved. The as-developed method was capable of differentiating NPC stages by the level of mir-205 quantified in serum with only 10 μL of serum and the whole assay can be completed in an hour. The experimental results agreed well with reported and while the quantity of mir-205 determined by our assay was found comparable to that of quantitative reverse transcription polymerase chain reaction (qRT-PCR), supporting that this assay can be served as a promising non-invasive detection tool for early NPC diagnosis, monitoring and staging.

In chapter 4, a self-assembled protein nanofibril based online pre-concentrating sensor was developed. This solution-based hybridization assay was applied to quantified the amount of target miRNAs, mir-196a. Biotinylated locked nucleic acid (LNA) of complimentary sequence was served as the probe to capture the target miRNA analyte. The target hybridization duplex was immobilized on the backbone of the nanofibril through the biotin-streptavidin interaction. The quantification was achieved by the fluorescence intensity measured with total internal reflection fluorescence microscopy. A detection limit of 1 pM was achieved with trace amount of sample consumption. This assay showed efficient single-base mismatch discrimination. The applicability of quantifying circulating

mir-196a in both normal and cancer patient's serums was also demonstrated.

In chapter 5, a magnetic nanoparticles based sandwich immunosensor with carbazole-based cyanine as the fluorescence labeling dye for the direct quantification of prostate cancer related antigen, PSA, was developed. Taking benefit of the magnetic property of the nanoparticles, the target sandwich immunocomposites can be easily online separated from the sample matrix. The as-developed assay can efficiently discriminate the target PSA from other disease related antigens and achieve a LOD of 400 fM (13 pg/mL) and a LOQ of 2 pM (0.66 ng/mL). As the whole detection assay can be completed in 1 h with only 10 μL of sample, this assay is fast and cost effective and of high potential for early disease and cancer diagnosis, staging and monitoring.

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