SENSING OF LUNG CANCER USING SURFACE POTENTIAL

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Abstract- A label-free and high-sensitive sensing method of lung cancer mutations using surface potential and a gold nanoparticle (AuNP) is developed. The detection is based on the evaluation of the surface potential of each AuNP. When AuNPs are modified with probe DNA (AuNP–pDNA), the surface potential is shifted towards the negative potential due to the negatively charged DNA backbone. When AuNP–pDNA captures target mutant DNA through DNA hybridization, an additional surface potential shift occurs. The platform is able to sense lung cancer mutant DNA with a limit of detection (LOD) of 3.3 pM.

Keywords- lung cancer; surface potential; gold nanoparticle; probe DNA

I. INTRODUCTION

Lung cancer is one of the most common severe disease driving to the death of human. Lung cancer can be divided into two cases of small-cell lung cancer (SCLC) and non-SCLC (NSCLC), and about 80% of lung cancers belong to the case of NSCLC. From several studies, correlation between epidermal growth factor receptor (EGFR) and NSCLCs has been investigated[1]. Therefore, EGFR inhibitor drugs such as gefitinib and erlotinib have been used as lung cancer treatments. However, the treatments result showed low response (10~20%) in clinical trials due to EGFR mutations that cause the drug resistance[1]. Patients with resistance to EGFR inhibitor drugs usually are positive to KRAS mutation. Therefore, assessment of EGFR and KRAS mutation is essential for target therapies of NSCLC patient.

In order to overcome the limitation of conventional therapies, overall EGFR and KRAS mutations have to be monitored. In this work, only detection of EGFR will be presented. A variety of techniques have been presented for the detection of EGFR mutations. The standard detection method of EGFR mutation in ctDNA relies on real-time polymerase chain reaction (PCR)[2]. Real-time PCR method provides high sensitive detection performance. However, as the amplification step increases, cost effect and complexity increase as well. Other types of technology such as BEAMing, next generation sequencing (NGS), electrochemical sensor and silicon nanowire field-effect transistor have been presented. However, those technologies have limitations of low sensitivity, high cost and complexity of data analyzation. In this report, we propose a label-free and high-sensitive detection method of lung cancer using surface potential and a gold nanoparticle (AuNP) based platform.

The label-free and high-sensitive detection is achieved by using surface potential as well as AuNP platform that densely adsorb probe DNA and minimize the sensing area. The detection is performed based on the evaluation of surface potential of each AuNP using KPFM (see Fig. 1)

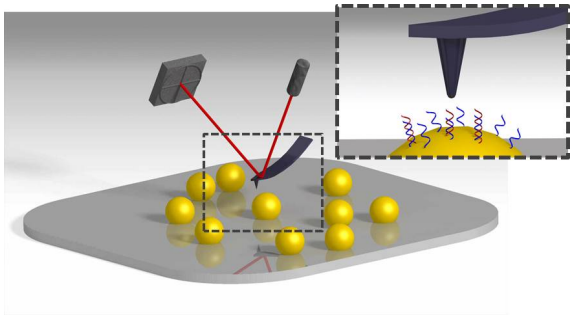


Fig. 1 Schematic of detection mechanism

II. MATERIALS AND METHODS

A. AuNP DNA modification

In this experiment, probe DNA of 1 μmol with sequences of 5'-thiol-ACGCCAACAGCTC-3' and 3'-thiol-GCGATAGTTCTGTAGAGGCT-5' were dissolved in a TE buffer. 1.5 μl of 10 mM TCEP solution were added to these probe DNA to activate the thiol-modified DNA, which was then incubated at room temperature for 1 hour, then added to 20 nm-diameter AuNPs in citrate buffer. The produced solution was stirred with a magnetic bar at room temperature in a dark room for 16h. The resulting functionalized AuNP was centrifuged at 16,000g at room temperature for 15 mins to discard supernatant free probe DNA, then the discarded amount was replaced with TE buffer. For confirmation of the functionalization of probe DNA on gold nanoparticles, solution of bare AuNP and modified AuNP were added to sodium chloride of 1mM. And then color changing of each solution was observed after 24h.

B. AuNP DNA sampling with ctDNA

Target DNA with sequences of 3'-TGCGGTTGTCGAG-5' and 5'-GGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGCTATCAAGGCATCTCCGAAAGCCAACAAGGAAATCCTCGAT-3' was inserted into several solutions with varying concentrations. The interaction time is 1 hour each. A single droplet (2 μl) of this solution was placed on a silicon substrate, washed by ethyl alcohol (99.9 %) and triple

distilled water (di-water), then was dried for 5 hours before usage in the desiccator. The functionalized AuNPs were absorbed by the substrate due to the van der waals force by this procedure.

CONCLUSIONS

A label-free and high-sensitive detection of lung cancer was performed on the AuNP platform. The high-sensitive detection of EGFR was achieved by the formation of densely adsorbed probe DNAs on AuNPs as well as the minimal sensing area due to the small diameter of AuNP, and label-free detection was performed due to the advantage of KPFM that measures a native property of DNA. The platform was able to detect 84 mer EGFR mutant DNA with LOD of 3.3 pM.

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