SENSING OF CANCER DNA USING QUARTZ CRYSTAL MICROBALANCE

Chanho Park[a], Hyunjun Park[a], Sungsoo Na[a]*

[a]*Korea university, Mechanical engineering, Seoul, Korea*
**email: nass@korea.ac.kr*

A label-free and high-sensitive sensing method of cancer DNA mutations using QCM(quartz crystal microbalance) is developed. The detection is based on the evaluation of the resonance frequecny. When probe DNA (pDNA) is introduced on quartz of sensor of QCM, the resonance frequency is shifted. When pDNA captures target mutant DNA through DNA hybridization, an additional resonance frequency shift occurs due to mass change in association with target DNA. The platform is able to sense EGFR, lung cancer mutant DNA with a limit of detection (LOD) of 1.0 nM.

Keywords: Epidermal growth factor receptor (EGFR), Quartz crystal microbalance (QCM), Nano-porous, Mutation, In situ detection

1. Introduction

Early lung cancer detection is very important issue because it is one of the most common diseases that causes death. After the discovery of the presence of nucleic acids circulating in the human blood in 1948 by Mandel and Metais [1], liquid biopsy become a novel, diagnostic and predictive tool for cancer detection. In previous studies, it has been shown that the circulating tumor DNAs (ctDNA) from cancer cells is found in blood in patients with advanced tumors. Therefore, ctDNAs enable the possibility of finding a profile of specific tumor metastasis, status and mutations [2].

There are two types of Lung cancer, small–cell lung cancer (SCLC) and non–SCLC (NSCLC). About 80% of lung cancer belongs to NSCLCs. It is well known that NSCLCs and epidermal growth factor receptor (EGFR) have some relation [3]. Because of the correlation, the status of EGFR mutations is very important factor of NSCLC diagnosis. Therefore, assessment of EGFR mutation is essential for targeted therapies of patients with NSCLC. EGFR mutations must be monitored to overcome the limitation of conventional therapies and diagnose NSCLC.

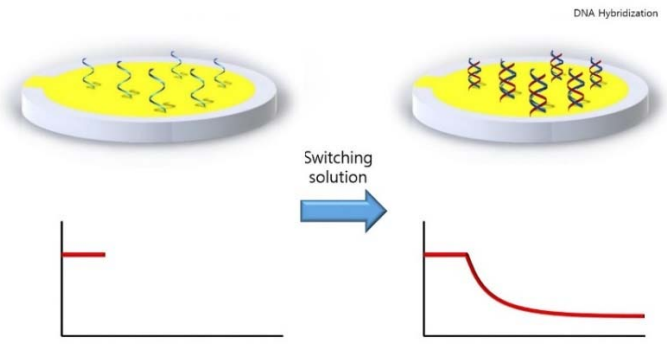

Figure 1: Schematic of EGFR mutation detection using quartz crystal microbalance.

Up to now, tissue biopsies are widely used to monitor EGFR mutation. However, the tissue biopsies, also called solid biopsies, have several limitations. These biopsies are very invasive

methods and cannot monitor the ctDNAs in real-time. Also, the major drawback of solid biopsies is that it does not represent the all mutations of cancer patients.

Recently, liquid biopsies have been developed to overcome the limitations of solid biopsies. Liquid biopsies are non-invasive approach that can detect EGFR mutations in metastatic cancer patients in real-time [4].

In this study, we provide a method to detect EGFR mutations using Quartz Crystal Microbalance(QCM). Mass and frequency is direct relationship. QCM is a widely used biosensor based on the relationship. [5]. As shown in figure 1, when the target DNAs hybridize with the probe DNAs, the total mass of the QCM electrode increases and the resonance frequency of the electrode decreases [6, 7]. This phenomenon was used to detect in situ EGFR mutations. We achieved the detection of EGFR mutation at the detection limit of 1 nM (LOD).

2. Method

2.1 Probe DNA immobilization on QCM electrode

Probe DNAs (pDNAs) are immobilized on a QCM electrode using a gold-thiol bond. First, to activate the thiolated probe DNAs, the TCEP solution is added to the pDNAs solution. After 3 h, the solution is dissolved in 10 mM TE buffer. The final concentration of probe DNA is 10 μM. Drop the probe DNA solution on the QCM electrode for about 2 hours and rinse with distilled water.

2.2 EGFR ctDNA mutations detection

In-situ detection was performed using the SRS QCM200. QCM electrode with probe DNA mounted to QCM flow cell and EGFR mutant DNA was dissolved in 10 mM NaCl solution. The control solution (distilled water with 10 mM of NaCl) flowed into the flow cell. After the resonance frequency has saturated (about 5 min), switch the solution to the EGFR mutation DNA solution. The flow rate of the solution was 6 ml / h.

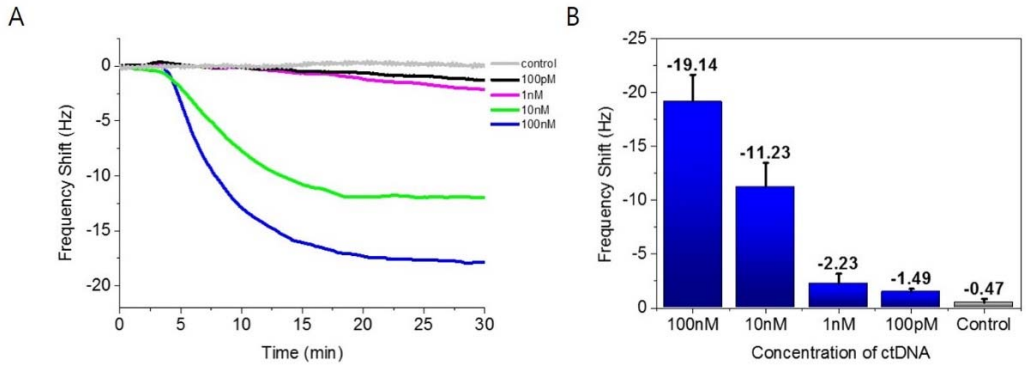

Figure 2: A. Real time graph of resonance frequency shift of EGFR mutant DNA detection with respect to time at various DNA concentrations. B. Average resonance frequency shift obtained from repeated experiment with respect to EGFR mutant DNA concentration at 30 min.

3. Result and discussion.

After we observed that the resonance frequency was maintained constant for about 5 minutes after the control solution was added to the QCM device, we flowed the EGFR mutation solution.

When solution is converted to an EGFR mutant DNA solution, resonance frequency changes were confirmed (Figure 2A). The resonance frequency converges to a constant value at about 30 minutes after the flow switch. The final frequency shift values were different varied to the EGFR mutation concentrations. The high frequency shift was observed at high EGFR mutant DNA concentration. The obtained resonance frequency shift values were -19.14 ± 2.51, -11.23 ± 2.24, -2.23 ± 0.95, -1.49 ± 0.28, and -0.47 ± 0.38 Hz, respectively, at 10^2, 10^1, 10^0, 10^{-1} and 0 (control group) nM (Figure 2B). From these values we have determined the detection limit of detection of this experiment to be 1 nM.

4. Conclusion

We performed the detection of high sensitive EGFR mutation using QCM. This detection technique is based on the mass change and the resonance frequency change caused by the complementary binding of the target DNA to the probe DNA attached on the electrode. Real-time detection of EGFR mutation DNA was performed using QCM in a very simple way. The detection limit of this detection method is 1 nM. Our proposed method is very sensitive and will enable the diagnosis of EGFR mutation in lung cancer patients.

5. Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) under Grant Number NRF-2007-0056094 and NRF-2014R1A2A1A11052389 which are funded by the Ministry of Science, ICT & Future Planning.

REFERENCES

1 Mandel, P. and P. Metais, *Nucleic acids in human blood plasma.* CR Acad Sci Paris, 1948. **142**: p. 241-243.

2 Crowley, E., et al., *Liquid biopsy: monitoring cancer-genetics in the blood.* Nat Rev Clin Oncol, 2013. **10**(8): p. 472-484.

3 Jang, K., et al., *Label-free and high-sensitive detection of Kirsten rat sarcoma viral oncogene homolog and epidermal growth factor receptor mutation using Kelvin probe force microscopy.* Biosensors and Bioelectronics, 2017. **87**: p. 222-228.

4 Heitzer, E., P. Ulz, and J.B. Geigl, *Circulating Tumor DNA as a Liquid Biopsy for Cancer.* Clinical Chemistry, 2015. **61**(1): p. 112-123.

5 Ferreira, G.N.M., A.-C. da-Silva, and B. Tomé, *Acoustic wave biosensors: physical models and biological applications of quartz crystal microbalance.* Trends in Biotechnology, 2009. **27**(12): p. 689-697.

6 Sauerbrey, G., *The use of quartz crystal oscillators for weighing thin layers and for microweighing applications.* 1991.

7 Sauerbrey, G., *Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung.* Zeitschrift für Physik, 1959. **155**(2): p. 206-222.