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AN INTEGRATED MICROFLUIDIC PLATFORM TO DETECT FXYD2 RNA EXPRESSION IN ASCITES FOR DIAGNOSIS OF OVARIAN CLEAR CELL CARCINOMA

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Abstract

An integrated microfluidic platform was developed to automate the entire procedures from cancer cell capturing in ascites, RNA extraction, and on-chip quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for diagnosis of ovarian clear cell carcinoma (OCCC), which is a subtype of ovarian cancer (OvCa) with high mortality. FXYD2 gene expression was found highly associated with OCCC. A new protocol for detecting FXYD2 gene expression by referencing housekeeping gene was realized on the integrated microfluidic system. This detection method can be applied for diagnosis or prognosis monitoring of OCCC based on detection of the target gene expression level in ascites.

Introduction

Ovarian cancer was a serious gynecologic cancer with a high mortality rate due to its undetectable early-stage properties. However, current methods cannot guarantee fast and accurate diagnosis. High specificity and affinity reagents are therefore needed for early and accurate detection of OvCa.

FXYD2 gene coding the Na^+/K^+-ATPase subunit was found upregulated in OvCa and associated with OvCa tumorigenesis, particularly in OCCC, a high-mortality OvCa subtype. Molecular analysis could give a novel approach for diagnosing FXYD2 expression levels. This work used a new microfluidic device to automate the entire process from ascites of patients and a qRT-PCR process targeting FXYD2 to examine oncogenic quantity of tumor cells.

A new platform was developed to automate cancer diagnosis starting from cell capture to qRT-PCR for detecting FXYD2 expression in ascites. An efficient probe assay with a fluorophore and quencher was used to guarantee amplification of the target gene. Finally, a simple Ct ratio index was used to differentiate the degree of gene expression of cells in samples. Overall, this platform allows the detection of the expression level of the target gene using liquid samples.

Methodology

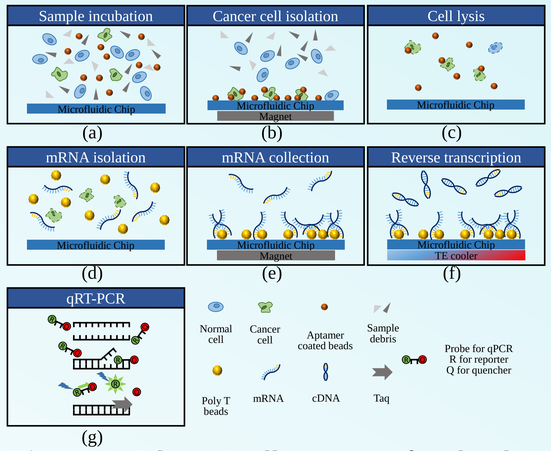

Figure 1. *Schematic illustration of molecular diagnosis of OCCC on the integrated microfluidic system, including cancer cell isolation, RNA isolation and qRT-PCR.*

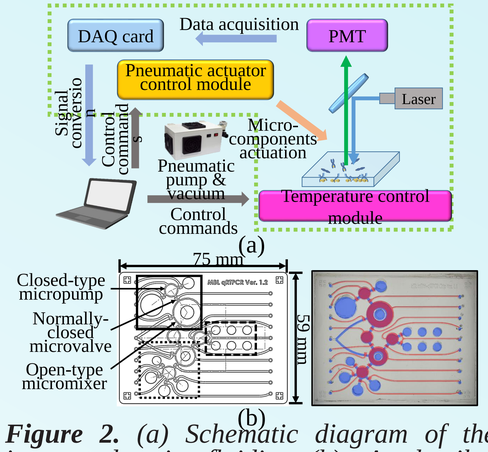

Figure 2. *(a) Schematic diagram of the integrated microfluidic. (b) A detailed design (left) and a photograph (right) of the chip which was equipped with micromixers, microvalves and micropumps*

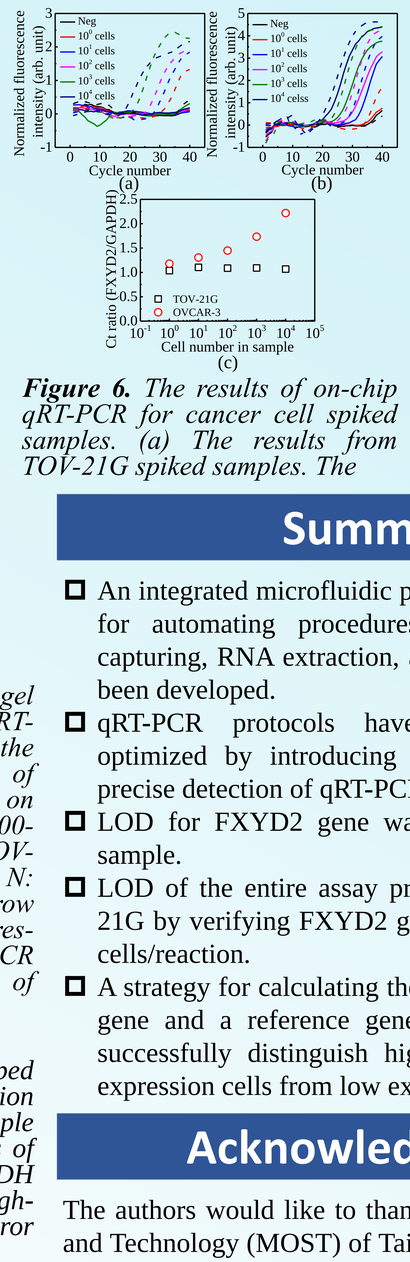

Figure 6. *The results of on-chip qRT-PCR for cancer cell spiked samples. (a) The results from TOV-21G spiked samples. The raw data of fluorescent signals in each PCR cycles. FXYD2 (solid line); GAPDH (dash line). (b) The same data presentation for OVCAR-3 cells spiked samples. (c) The Ct ratios of FXYD2/GAPDH for TOV-21G and OVCAR-3 cells. Ct ratios for high FXYD2 expression cell (TOV-21G) are below 1.25. Ct ratios for low or non- expression cell (OVCAR-3) are above 1.25.*

Results

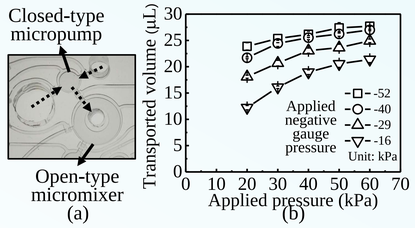

Figure 3. *Performance of the micropump. (a) A photograph of the micropump. (b) The relationship between the transport liquid volume and the applied negative gauge pressure.*

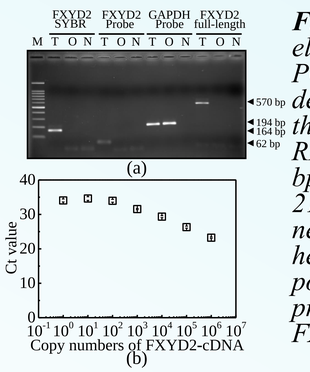

Figure 4. *Agarose gel electrophoresis for qRT-PCR products and the detection performance of the qRT-PCR protocol on RNA detection. (a) M: 100-bp DNA ladders; T: TOV-21G; O: OVCAR-3; N: negative control. The arrow heads indicate the corresponding size of PCR products. (b) The LOD of FXYD2 copy number.*

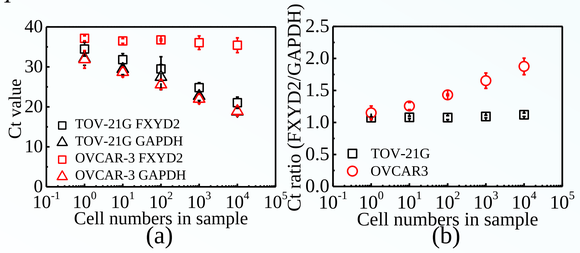

Figure 5. *The performance of the developed method and LOD for on-bench RNA detection protocol with qRT-PCR. (a) The Ct for the sample of TOV-21G and OVCAR-3 with 10-fold series of dilutions. (b) The Ct ratio of FXYD2/GAPDH showed the significant difference between high-expression cells and low-expression cells. All error bars indicated standard ($n \geq 3$).*

Summary

- An integrated microfluidic platform for gene detection for automating procedures including cancer cell capturing, RNA extraction, and on-chip qRT-PCR has been developed.
- qRT-PCR protocols have been improved and optimized by introducing probe primers for more precise detection of qRT-PCR amplicons.
- LOD for FXYD2 gene was around 10^3 copies per sample.
- LOD of the entire assay process for detecting TOV-21G by verifying FXYD2 gene could be as low as 10 cells/reaction.
- A strategy for calculating the Ct ratio between a target gene and a reference gene has been developed to successfully distinguish high target gene (FXYD2) expression cells from low expression cells.

Acknowledgement

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