A MICROFLUIDIC PLATFORM FOR DIAGNOSIS OF OVARIAN CLEAR CELL CARCINOMA VIA QUANTIFICATION OF FXYD2 GENE

Ting-Hang Liu[1], Chang-Ni Lin[2, 3], Keng-Fu Hsu[2, 3] and Gwo-Bin Lee[1, 4, 5*]

[1]Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan
[2]Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, College of Medicine, Tainan, Taiwan
[3]Graduate Institute of Clinical Medicine, National Cheng Kung University Hospital, College of Medicine, Tainan, Taiwan
[4]Institute of Biomedical Engineering, National Tsing Hua University, Hsinchu, Taiwan
[5]Institute of Nano Engineering and Microsystems, National Tsing Hua University, Hsinchu, Taiwan

ABSTRACT

Ovarian cancer (OvCa), with an extremely low 5-year survival rate, is difficult for early diagnosis and prognosis monitoring due to poorly sensitive and specific cancer biomarkers for detection. Furthermore, ovarian clear cell carcinoma (OCCC), a subtype of epithelial ovarian cancers, is recognized as an aggressive and high-grade neoplasm. Hence, early detection and molecular classification of OCCC for targeted therapies is required to improve prognosis. In this work, an integrated microfluidic system capable of isolation of OvCa cells from liquid biopsy (such as ascites) via aptamer-coated magnetic beads. The following RT-qPCR for quantification of the FXYD2 gene was developed, which encodes the γ subunit and enzymatic modulators of the Na^+/K^+ ATPase and could be up-regulated in OCCC than other types of ovarian cells. Experimental results showed that a limit of detection could be as low as 10 cells/reaction, which is satisfactory for future clinical trials.

EXPERIMENTAL PROCESS

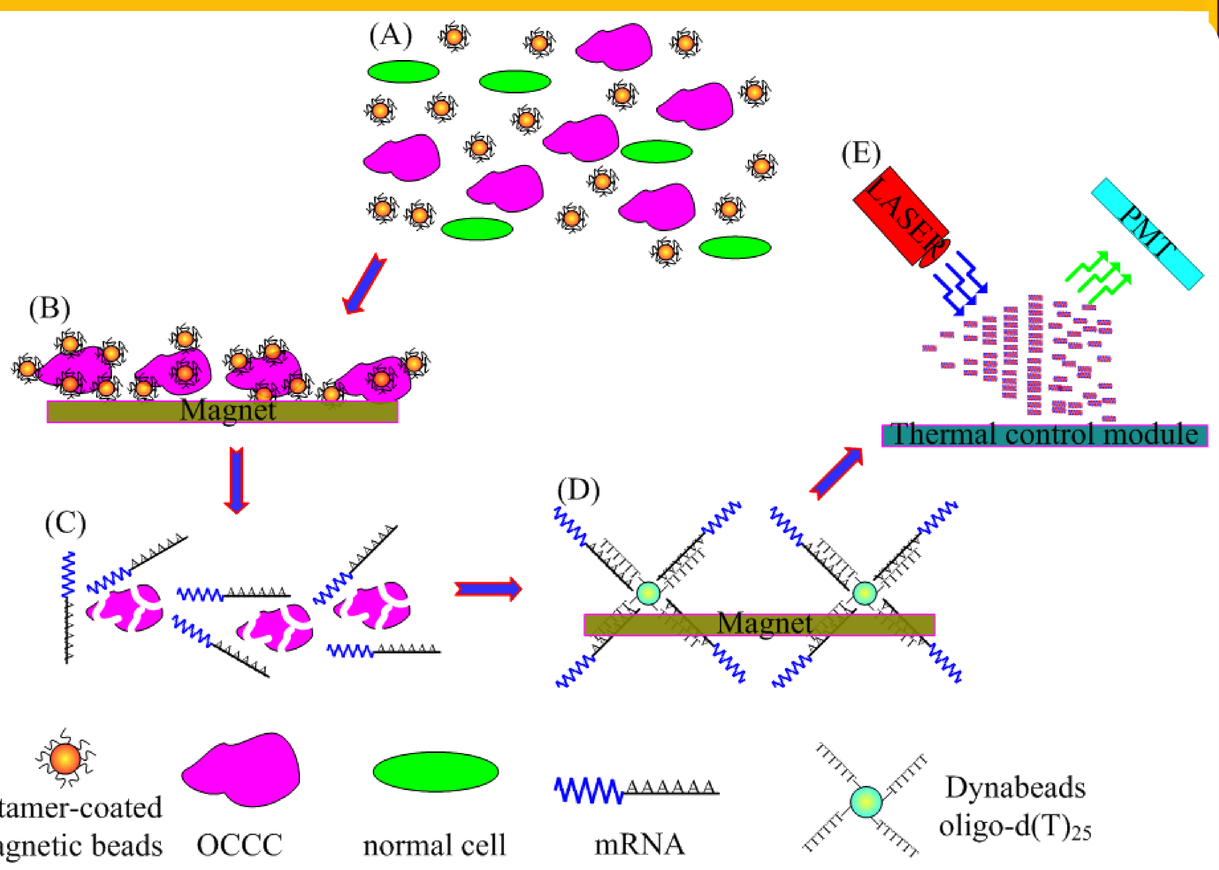

Figure 1. (A) OCCC cells in ascites were captured by aptamer-coated magnetic beads; (B) OCCC isolation under a magnetic field; (C) Chemical lysis of captured OCCC to release RNA; (D) mRNA isolation by using Dynabeads oligo-d$(T)_{25}$; (E) Thermocycling for reverse-transcription to generate cDNA from mRNA and then cDNA was used as a template for the following RT-qPCR. The PCR products were optically detected by using a laser-induced fluorescent system.

INTEGRATED MICROFLUIDIC SYSTEM and CHIP

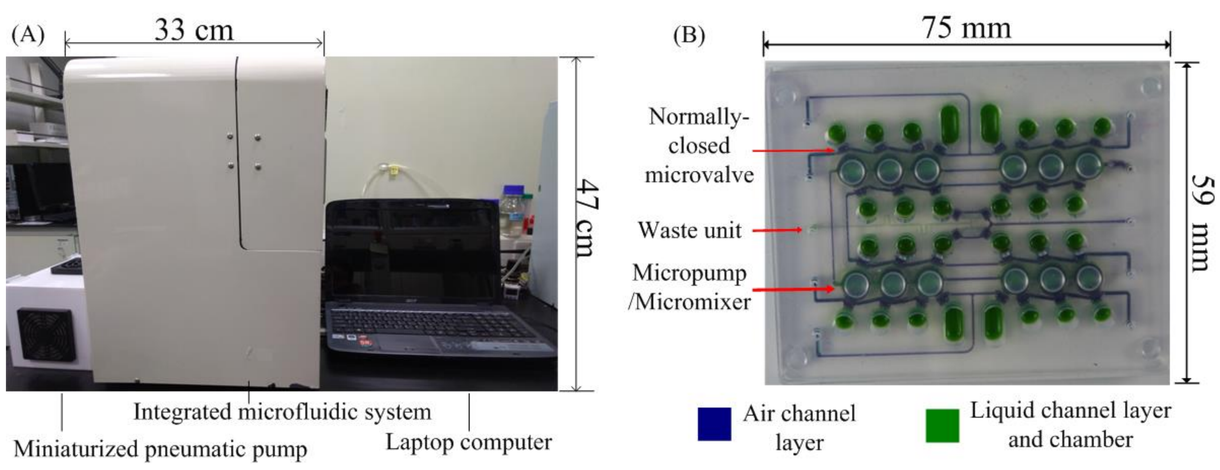

Figure 2. Experimental setup of the integrated microfluidic system (A) and a photograph of the microfluidic chip (B) equipped with micropumps, microvalves and micromixers to automate the entire process.

RESULTS

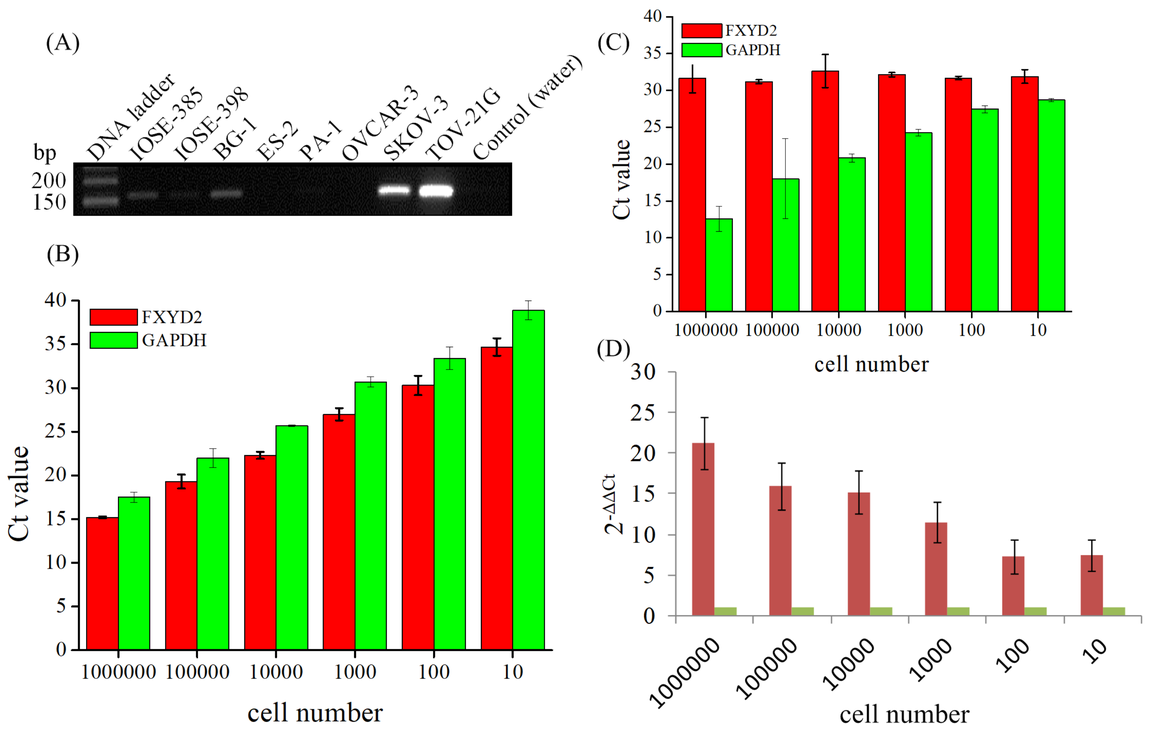

Figure 3. (A) FXYD2 expression level of different ovarian (cancer) cells. SKOV-3 and TOV-21 are OCCC cell lines with an obvious FXYD2 gene expression level higher than the other cell lines. The Ct values from RT-qPCR for FXYD2 and GAPDH gene expression level in various TOV-21G (2B) and OVACAR-3 (2C) cells respectively. (D) The relative expression level of FXYD2/GAPDH in TOV-21G and OVACAR-3 cells calculated from 2-Ct. The LOD of FXYD2 gene expression level of TOV-21G with 10-fold series of dilutions

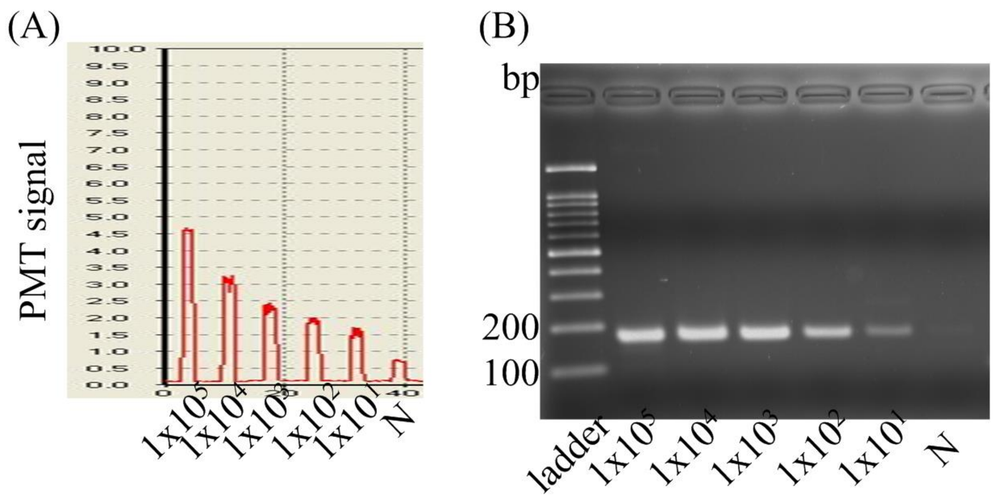

Figure 4. (A) Fluorescent signals detected by the integrated microfluidic system; (B) PCR products were further analyzed by agarose-gel electrophoresis for comparison. ladder: 100-bp ladders; N: negative control (distilled water); Lanes 1-5 represented the TOV-21G with cell numbers ranging from 10^5-10 cells/reaction.

CONCLUSIONS

1.We have developed a microfluidic system for detection of OCCC via quantification of FXYD2 gene.

2.Experimental data showed that the limit of detection of this developed molecular diagnosis could be as low as 10 cells/reaction.

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國立清華大學 NATIONAL TSING HUA UNIVERSITY

國立成功大學附設醫院 National Cheng Kung University Hospital