AN INTEGRATED MICROFLUIDIC SYSTEM FOR SCREENING OF APTAMERS SPECIFIC TO COLON CANCER CELLS AND STEM CELLS
BY UTILIZING ON-CHIP CELL-SELEX

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ABSTRACT
Colon cancer is the most frequently diagnosed cancer, taking about 700,000 lives every year. In stage
I, colon cancer shows the five-year survival rate higher than 90%. However, after medical therapy, there
are still a group of cancer stem cells (CSCs, less than 0.1 %) hard to treat. But there are no specific
biomarker only presented to recognize colon-CSCs. In this study, a new microfluidic system was
demonstrated to be able to select high-affinity aptamers specific to colon-CSC and colon cancer cells by
performing an automated on-chip Cell-SELEX process.

KEYWORDS: Colon cancer stem cell, Microfluidic, SELEX, Aptamer

INTRODUCTION
Traditional methods for colon cancer diagnosis are majorly invasive methods such as proctoscopy,
flexible sigmoidoscopy, colofibroscopy et al, but these scope-based methods can make patients
discomfort and bowel preparation needed [1]. After diagnosis and proper medical therapy, there are still
very few cancer stem cells (CSCs, less than 0.1 %) hard to treat, which are a tiny group of cancer cells
having the ability of self-renewal, multiple lineages development, and extensively proliferation, may play
an important role for cancer relapse [2]. In order to detect and isolate colon-CSCs, CD44 has been used as
biomarkers of colon-CSC. However, CD44 is not specific enough and also presents in other types of
CSCs [3]. This study therefore presents a new integrated microfluidic system for continuous selection of
aptamers specific to the colon-CSC and colon cancer using a cell-based systematic evolution of ligands
exponential enrichment (Cell-SELEX) process.

Figure 1: Illustration of the CSC Cell-SELEX process.
EXPERIMENTAL

The working process of CSC Cell-SELEX is schematically shown in Figure 1 while the colon-CSCs and colon cancer cell line (HCT-8) were used for specific aptamers screening. Briefly, single-stranded DNA (ssDNA) library was incubated with target cells for positive selection (Fig. 1(a-1)). After washing, ssDNA-target cell complex were thermally lysed to release ssDNA (Fig. 1(a-2)). Released ssDNA was further incubated with control cells for negative selection (Fig. 1(a-3)). Then incubation supernatant was collected to amplify ssDNA by using polymerase chain reaction (PCR) process (Fig. 1(a-4)). These amplified ssDNA were used for next round of Cell-SELEX screening (Fig. 1(a-5)) and the legends indicating the different symbols were shown in Figure 1(b).

An integrated microfluidic chip was designed to perform the entire process of CSC cell-SELEX automatically and showed in Figure 2, containing with one sample transportation unit, two reagent loading chambers (for the washing buffer and the binding buffer), normally-closed microvalves, two target cell chambers, two control cell chambers, two PCR reaction chambers, and two serpentine-shape micropumps (S-shape micropump).

![Figure 2: The integrated microfluidic system equipped with multiple micro-elements, four open-chamber micromixers and two serpentine-shape micropumps.](image)

RESULTS AND DISCUSSION

Enrichment cultured colon-CSCs and HCT-8 cells were used as target cells and control cells. As shown in Figures 3, anti-CD44 fluorescence immuno-staining analysis with CR-CSCs after enrichment

![Figure 3: CD44 fluorescence antibody analysis with (a) colon cancer cells (HCT-8) and (b) colon cancer stem cells (colon-CSCs) after enrichment suspension culture.](image)
suspension culture and HCT-8. These fluorescence images of HCT-8 and CR-CSC were indicated that only CR-CSCs presented red fluorescence signal significantly, indicating that these cells expressed abundant CD44 proteins and had properties of CSCs.

After five rounds of on-chip screening, the flow cytometric analysis was applied to confirm the screening results. Furthermore, one aptamer has the ability to specific recognize colon-CSC was selected, as Figure 4(a) shown, binding analysis by green fluorescence modified aptamers with colon CSCs. Figure 4(b) show that dissociation constant \(K_d\) analysis of CSC-35 aptamer and the \(K_d\) value was measured to be \(44.8 \pm 10.2\) nM, which is comparable to antibody.

**CONCLUSION**

In this study, a new microfluidic system was demonstrated to be able to select high-affinity aptamers specific to CR-CSCs and HCT-8 cell line by performing an automated on-chip Cell-SELEX process. anti-CD44 antibody was firstly applied to confirm the characterization of suspension cultured CR-CSCs. One CR-CSC-specific aptamer was successfully selected, showing high affinities towards CR-CSC with \(K_d\) of 44.8 nM, which is comparable to antibodies. This developed technique may be promising for screening of biomarker for these cancer cells, which could be useful for early diagnosis of CR-CSC/CRC or even target therapeutics. In the future, it may be applied in personalized medicine for aptamer screening or drug discovery.

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