DEVELOPMENT OF SPECIFIC APTAMERS WITH DIFFERENT HISTOLOGICAL CLASSIFIED OVARIAN CANCER CELLS BY UTILIZING ON-CHIP OVCA CELL-SELEX

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ABSTRACT

Ovarian cancer is the second most common type of gynecological cancers. Comparing with the worst gynecological cancer, cervical cancer, the prognostication of ovarian cancer is relatively poor. It is challenging to diagnose it in the early stage where proper treatments are most effective. In order to achieve early detection of ovarian cancer, biomarkers with high specificity and affinity is therefore important. In this study, an integrated microfluidic system was developed for the multiple-cell-line and high-throughput screening of OvCa cell-SELEX automatically. An aptamer specific to ovarian cancer cells were successfully screened.

KEYWORDS: Aptamers, Different histology, Microfluidics, On-Chip Cell-SELEX, Ovarian cancer (OvCa)

INTRODUCTION

Ovarian cancer (OvCa) is the fifth common cancer in women around the world and about 200,000 of women have been diagnosed with ovarian cancer every year. An in vitro screening process, cell-based systematic evolution of ligands by exponential enrichment (Cell-SELEX) has been used for the selection of OvCa specific aptamers by using bench-top apparatus [1]. Furthermore, an automatic microfluidic system for cancer stem-like cell aptamer screening was also developed by our group [2]. However, different histological types of OvCa cells may be different in genetic origins and further clinical performances. Different histological OvCa cells can even show different sensitivity or resistance to various therapy drugs [3]. Therefore, it is important to screen aptamers which may be specific to different OvCa cells.

The working process of on-chip OvCa Cell-SELEX using magnetic beads is schematically shown in Figure 1. Briefly, it involves with the following processes, including (a) mixing ssDNA with target cells, (b) applying a magnetic force to collect the ssDNA-target cell complex and washing out the unbound ssDNA, (c) thermal lysis of target cells and then releasing bound ssDNA, (d) mixing ssDNA with control cells, (e) applying a magnetic force to collect the ssDNA-control cell complex and collecting the unbound ssDNA as aptamer candidates, (f) performing polymerase chain reaction (PCR) for next round of OvCa cell-SELEX screening, as shown in (g). However, this complexity of this screening process makes it extremely challenging to manipulate multiple cell lines in one time.

EXPERIMENTAL

Ovarian cancer cells could be classified in four major different histological cell types, including serous type, clear cell type, endometrioid type and mucous type. In this study, different histological types of OvCa cell lines were applied for cell-SELEX screening, as listed in Table 1. Figure 2 shows microscopic images of different types of OvCa cell lines; TOV21G was tightly adhesive; BG-1 was smaller cells but more sharp in cellular edges; TOV112D showed faster growth with bright and round-shape cells and IRGOV1 was observed to be a more aggregated cellular form than other three cell lines. In order to realize the multiple-cell-line and high-throughput screening of OvCa cell-SELEX, an integrated microfluidic system was designed and fabricated for automatic screening of aptamers.

![Figure 1: Schematic illustration of the OvCa cell-SELEX process in a magnetic bead-based system.](image-url)
As shown in Figure 3(a), the developed microfluidic system was equipped with one major transportation unit, one target cell region, one control cell region, and PCR chambers for final ssDNA amplification. Figure 3(b) shows a photograph of the microfluidic system on a customer-made dual-temperature control system. Note that the dual-temperature control system allows the control cells to keep around 10.3°C as the target cells are thermally lysed at 94.1°C, as shown in Fig. 3(c).

Table 1. Applied cell lines in this on-chip OvCa cell-SELEX

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Histology/Feature</th>
<th>Stage/Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOV21G</td>
<td>Clear cell type</td>
<td>III, ATCC-CRL11730</td>
</tr>
<tr>
<td>BG-1</td>
<td>Serous type</td>
<td>III</td>
</tr>
<tr>
<td>TOV112D</td>
<td>Endometrioid type</td>
<td>IIIC, ATCC-CRL11731</td>
</tr>
<tr>
<td>IRGOV1</td>
<td>Endometrioid type</td>
<td>III</td>
</tr>
</tbody>
</table>

Figure 2: Microscopic images of different histological OvCa cell lines.

RESULTS AND DISCUSSION

We used TOV21G and BG-1 as target cell and control cell, respectively for preliminary testing of the developed microfluidic system. Note that only $1\times10^4$ cells were required for this Cell-SELEX, which was much less than previous study [2]. Figure 4(b) shows the PCR signals of the on-chip OvCa Cell-SELEX. It was found that the PCR signals were still stable after 15 consecutive rounds of screening, indicating that the saturation of ssDNA-cell binding. The PCR product of the 15th round was then cloned by using a TOPO TA cloning kit. A competitive test using free TOV21G as a competitor during the test was then performed, as shown in Fig. 4(a). The screened aptamer exhibited a high affinity and specificity (Fig. 4(b)). Then two colonies in Fig. 4 (c) (colonies 17 and 19) were concluded to be specific to TOV21G cells.

Figure 5(a) is the flow cytometric analysis with BG-1 and TOV21G, in the Cy3 and Cy5 probing samples. The fluorescence intensity of TOV21G target cells, the Cy5 signal, majorly shifted from $10^2$ to $10^3$, indicating that these screened aptamers exhibited a specific affinity with TOV21G cells. Figure 5(b) is the 2D structure prediction of TOV-17 and 19 by using mfold software, version 3.5 (http://mfold.rna.albany.edu/). Furthermore, the screened aptamers were sequenced and the sequences of the screened aptamers were shown as follows.

CX_TOV17C: $5'-$

TGTCGGTGTCTGGTGATGCGCAATCTAGAGAAAGAATATAGTGGAAACACAGGCGCACAACACAGAAGGACG

G-3’.
Further testing regarding the quantitative measurement of association constant about the screened aptamers is undergoing.

**Figure 4:** (a) Schematic illustration of the competitive test by using a magnetic bead-based method, (b) cell-SELEX screening in 15 rounds, and (c) the competitive test resulting of colonies 17 and 19.

**Figure 5:** (a) flow cytometric analysis with BG-1 and TOV21G, and (b) 2D structure prediction with mfold program.

**CONCLUSION**

When compared with the previous study [2], the process steps in the developed microfluidic system were shortened from traditional 22 working runs to 15 on-chip rounds. Furthermore, less applied cell number, from $5 \times 10^5$ to $1 \times 10^4$ cells, were required for Cell-SELEX. More importantly, an automatic screening system makes this study capable of performing multiple-cell-line and high-throughput screening on one integrated microfluidic system. It may be further applied for personal aptamer screening or drug discovery in the near future.

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**REFERENCES**


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