A NOVEL METHOD TO INVESTIGATE PROTEOMIC PROFILING OF CANCERS USING A MICROFLUIDIC IMMUNOHISTOCHEMISTRY SYSTEM

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ABSTRACT

This paper presents a massively multiplexed and quantitative immunohistochemistry (IHC) platform using microfluidics technology. The device was fabricated with two-step multiplayer soft lithography and a microvalve was integrated to simultaneously flow 10 biomarkers into individual reaction channel. The microfluidic massive IHC system enabled 20 IHC assays on a biological specimen at the same time, saving 16-fold time consumption. Moreover, the expressions of the biomarkers were quantitatively compared. The immunohistochemical staining from the microfluidic system showed accurate localization and comparable quality with that of conventional IHC method. Proteomic profiling was examined for 4 breast cancer cell lines, AU-565, HCC70, MCF-7 and SK-BR-3. The microfluidic massive IHC system is expected to be useful for accurate histopathological diagnosis using numerous specific biomarkers at a time.

KEYWORDS: Immunohistochemistry, Multiplexed diagnosis, Proteomic profiling, Breast cancer

INTRODUCTION

The characteristic of heterogeneity in breast cancer has been regarded as one of the most principal reasons prohibiting complete cure [1]. Discovery of novel biomarkers makes it possible subclassification of the heterogeneous cancers, so that patient-centered therapy has been gradually progressed by molecularly targeted approaches. Immunohistochemistry (IHC) has been a major pathological diagnostic method in various malignancies and many studies showing the relationship between immunohistochemical profiles and molecular classification support that IHC can be a significant role in personalized medicine of breast cancer patients [2]. However, conventional IHC method is not adequate for examination of multiple biomarkers since it requires consumption of many tissue slices and proportionally increases diagnostic cost which can give a big financial burden to patients. Moreover, current qualitative decision for scoring is also one of the big challenges toward complete cure of cancers.

Therefore, the development of innovative multiplexing IHC method satisfying not only investigation of multiple biomarkers with reducing consumption of tissue slice but also quantification for the biomarkers is very important task to better understand the status of cancer patients.

Figure 1. Configuration of the microfluidic IHC platform. (A) Design of the microfluidic device. The device was designed to screen ten biomarkers on a sample at the same time. (B) The schematic of an assembler for reversible sealing between a sample slide and a microfluidic IHC device. (C) The schematic of multiplexed IHC staining.
EXPERIMENTAL

The preparation of cell blocks and tissues was specifically described in our previous work [3]. Brief procedure is that HCC70, MCF-7, and AU-565 were maintained in RPMI-1640 medium and SK-BR-3 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 IU ml⁻¹ penicillin, and 100 mg ml⁻¹ streptomycin. To make a cell block, the harvested cells were centrifuged, fixed in formalin, suspended in agar, and embedded in paraffin to produce a cell block. Paraffin-embedded cell blocks were sectioned with 4 µm thickness. The sections were mounted and baked onto positively charged slides, and they were performed to dry for 1 h at room temperature, followed by 1 h in a convection incubator at 60°C.

To fill the biomarkers into individual reservoirs and react biomarkers with a target sample at the same time, microvalves were required. Therefore, rounded channel was fabricated for microvalves and rectangular channel was also created to form even fluid profile in width of the reaction channels. The fluidic channel mold of the device was fabricated by two step multilayer soft lithography. To construct rectangular reaction channels, SU-8 2025 was spincoated to make 60 µm thickness on a bare silicon wafer. To make round-shaped remnant fluidic channel, AZ 9260 was spincoated to make 20 µm thickness on the wafer.

Ten biomarkers known as predictive and prognostic indicators in breast cancers were used in this study and all biomarkers were 0.25-fold diluted compared to the antibody concentration of conventional IHC method. Detailed information for antibodies is described in Table 1.

Table 1. Biomarkers of breast cancers used in this study and their conditions.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Origin</th>
<th>Clone</th>
<th>Dilution factor</th>
<th>Working concentration in QIHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMM-HC</td>
<td>DAKO</td>
<td>SMMS-1</td>
<td>×200</td>
<td>×0.25</td>
</tr>
<tr>
<td>CK5</td>
<td>Novocastra</td>
<td>XM26</td>
<td>×50</td>
<td>×0.25</td>
</tr>
<tr>
<td>CK14</td>
<td>Novocastra</td>
<td>LL002</td>
<td>×100</td>
<td>×0.25</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Transduction</td>
<td>36</td>
<td>×250</td>
<td>×0.25</td>
</tr>
<tr>
<td>p53</td>
<td>VENTANA</td>
<td>Bp53-11</td>
<td>×1</td>
<td>×0.25</td>
</tr>
<tr>
<td>ER</td>
<td>VENTANA</td>
<td>6F11</td>
<td>×1</td>
<td>×0.25</td>
</tr>
<tr>
<td>p63</td>
<td>DAKO</td>
<td>4A4</td>
<td>×50</td>
<td>×0.25</td>
</tr>
<tr>
<td>PR</td>
<td>VENTANA</td>
<td>1A6</td>
<td>×1</td>
<td>×0.25</td>
</tr>
<tr>
<td>HER2</td>
<td>DAKO</td>
<td>polyclonal</td>
<td>×1000</td>
<td>×0.25</td>
</tr>
<tr>
<td>Ki-67</td>
<td>DAKO</td>
<td>MIB-1</td>
<td>×50</td>
<td>×0.25</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

A novel microfluidic multiplexed and quantitative IHC platform was developed to investigate proteomic profiling of breast tumors (Figure 1A and 1B). Fluidic resistance was considered for equivalent flow rate of individual reaction channel and individual reagent was flowed along each microchannel without cross-contamination (Figure 2A). After aligning the microfluidic device with a cell block, the massive multiplexed IHC process was conducted (Figure 2B) and twenty immunohistochemical assays were proceeded on a sample (Figure 3A). As shown in Figure 3B, the staining pattern was corresponded to the geometry of the reaction channel and each channel showed uniform staining for the cell block. In addition, the microfluidic IHC platform was applied to four breast cancer cell lines (MCF-7, SK-BR-3, AU-565, and HCC70) and the staining quality from the new method was comparable to that from the conventional IHC method (data not shown).

Figure 2. Verification of fluid stream in reversible sealing condition. (A) Each dye was flowed along the individual reaction channel without any cross contamination. Scale bar; 1 mm. (B) Overall procedure of microfluidic IHC method.
Figure 3. Alignment of the microfluidic device with a SK-BR-3 cell block. (A) An image being aligned between the device and the cell block after blocking process. (B) An image that the microfluidic IHC process was finished. Both scale bars are 500 µm.

After taking images for biomarkers, image analysis was conducted for the quantification of biomarker staining. As shown in Figure 4, proteomic profiling for ten biomarkers could be quantitatively analyzed for every breast cancer cell line via IHC fashion. The platform was also applied to a breast cancer tissue sample and the results were corresponded to the results of conventional IHC method. Since all of the biomarker expressions were presented on a single cell block, it eliminated the unexpected variation that may arise from multiple IHC steps, meaning that more credible quantitative comparison might be possible between biomarkers’ expressions.

Figure 4. Quantitative proteomic profiling of ten biomarkers for four breast cancer cell lines. Different proteomic profiling was shown depending on each breast cancer cell line and all of the breast cancer cells expressed Ki-67 protein.

CONCLUSION
In summary, the microfluidic massive IHC platform was demonstrated to address the multiplexing and in situ quantification of IHC fashion. This microfluidic platform is expected to realize precise subtyping of cancer patients by the proteomic profiling even though the size of tumor samples acquired by operation has been getting smaller due to early detection and neoadjuvant therapy.

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REFERENCES

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