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Tumor cell characterization and classification based on cellular specific membrane capacitance and cytoplasm conductivity

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ABSTRACT

This paper reports a microfluidic system that enables the characterization of tumor cell electrical properties where cells were aspirated through a constriction channel (cross-section area smaller than that of biological cells) with cellular impedance profiles measured and translated to specific membrane capacitance ($C_{specific\ membrane}$) and cytoplasm conductivity ($\sigma_{cytoplasm}$). Two batches of H1299 cells were quantified by the microfluidic platform with different constriction channel cross-section areas, recording no differences with statistical significance ($p<0.001$) in both $C_{specific\ membrane}$ (1.63 ± 0.52 vs. 1.65 ± 0.43 μF/cm^2) and $\sigma_{cytoplasm}$ (0.90 ± 0.19 vs. 0.92 ± 0.15 S/m), and thus confirming the reliability of the microfluidic platform. For paired high- and low-metastatic carcinoma strains 95D ($n_{cell}=537$) and 95C cells ($n_{cell}=486$), significant differences in both $C_{specific\ membrane}$ (2.00 ± 0.43 vs. 1.62 ± 0.39 μF/cm^2) and $\sigma_{cytoplasm}$ (0.88 ± 0.46 vs. 1.25 ± 0.35 S/m) were observed. Statistically significant difference only in $C_{specific\ membrane}$ (2.00 ± 0.43 vs. 1.58 ± 0.30 μF/cm^2) was observed for 95D cells ($n_{cell}=537$) and 95D CCNY-KD cells with single oncogene *CCNY* down regulation ($n_{cell}=479$, CCNY is a membrane-associated protein). In addition, statistically significant difference only in $\sigma_{cytoplasm}$ (0.73 ± 0.17 vs. 1.01 ± 0.17 S/m) was observed for A549 cells ($n_{cell}=487$) and A549 CypA-KD cells with single oncogene *CypA* down regulation ($n_{cell}=597$, CypA is a cytosolic protein). These results validated the developed microfluidic platform for $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ quantification and confirmed the feasibility of using $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ for tumor cell classification.

1. Introduction

Single-cell electrical properties (e.g., specific membrane capacitance ($C_{specific\ membrane}$) and cytoplasm conductivity ($\sigma_{cytoplasm}$)) are important for understanding cellular functions and status (Morgan et al., 2007; Valero et al., 2010; Zheng et al., 2013). As label-free biophysical markers, cellular electrical properties have been used to classify cell types as preliminarily demonstrated for tumor cells (Chen et al., 2011; Cho et al., 2009; Coley et al., 2007; Han et al., 2006; Zhao et al., 2013), stem cells (Song et al., 2013) and blood cells (Du et al., 2013; Holmes and Morgan, 2010; Holmes et al., 2009; Zheng et al., 2012).

In the field of tumor cell classification, conventional techniques such as dielectrophoresis have been used to characterize $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ where cells attached to dielectrophoretic electrodes at specific frequencies were counted, and the number of attached cells was then translated to intrinsic electrical properties. Based on this technique, differences in $\sigma_{cytoplasm}$ and $C_{specific\ membrane}$ for MCF-7 cells and their multi-drug resistant derivatives were reported (Coley et al. 2007). Although powerful, this technique can only provide electrical properties based on batch testing (e.g., purified cell lines) and cannot quantify electrical properties at the single cell level, rendering tumor cell classification with different malignant levels being impossible.

With the development of microfluidics featured with typical dimensions in the range of 1–100 μm (El-Ali et al., 2006; Wootton and Demello, 2010), micro electrical impedance spectroscopy was proposed for cell classification at the single cell level (Cheung et al., 2010; Sun and Morgan, 2010; Valero et al., 2010). Han et al., (2006) used negative pressures to aspirate individual tumor cells on top of two micro electrodes, followed by impedance sweeping

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and characterization. Based on collected impedance data, electrical property differences in breast cancer cell lines (e.g., MCF-7, MDA-MB-231, MDA-MB-435 and MCF-10) and head and neck cells (e.g., 686LN vs. 686LN-M4e) (Cho et al., 2009) were reported. However, these techniques have limited throughput where reported cell numbers were roughly ten cells per cell type and cannot provide intrinsic electrical properties such as $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$.

To address these issues, Sun et al. aspirated single cells continuously through a constriction channel with proper sealing between elongated single cells and constriction channel walls, which effectively blocked electric field lines and enabled cellular electrical property characterization. Based on this technique, a breast tumor cell line and its multiple drug resistant counterpart (Chen et al., 2011) were distinguished based on electrical properties of hundreds of cells. However, this technique can only collect size-dependent electrical properties rather than intrinsic electrical parameters such as $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$.

To enable $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ quantification, previously, we modified the constriction channel based on microfluidic platform where cells were aspirated through the constriction channel with two-frequency impedance data sample. Based on the proposed equivalent circuit model, the measured impedance data were used to derive $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ (Zhao et al., 2012). However, in this platform, whether variations in the channel cross-section geometry would affect the measurement results of $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ was not explored.

In this study, we first evaluated the reliability of the microfluidic system by aspirating two batches of H1299 cells through constriction channels with cross-section areas of 100 μm^2 and 120 μm^2, respectively. No significant differences in both $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ were observed. Then paired high-metastatic and low-metastatic carcinoma strains 95D and 95C cells were tested with significant differences in both $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ which are recorded. Furthermore, electrical property differences with statistical significance only in $C_{specific\ membrane}$ for 95D cells and 95D CCNY-KD cells with single oncogene *CCNY* down regulation (CCNY is a membrane-associated protein) and only in $\sigma_{cytoplasm}$ for A549 cells and A549 CypA-KD cells with single oncogene *CypA* down regulation (CypA is a cytosolic protein) were observed.

This paper builds on previous publications (Huang et al., 2014; Zhao et al., 2012; Zhao et al., 2013), and is featured with the following interesting findings. From the perspective of technical development, it was shown that the microfluidic platforms with different constriction channel cross-section areas produced statistically identical results for the same tumor cell line, demonstrating the reliability of the microfluidic platform. From the perspective of cell classification, compared to previous studies on testing different cell lines from different patient sources with distinct genetic backgrounds, in this study, paired high- and low-metastatic tumor cell lines subcultured from the same patient source and tumor cell lines with single oncogenes under regulation were tested and classified, which are more clinically relevant. As the first demonstration of classifying tumor cells and their counterparts with single oncogenes under regulation based on $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ from hundreds of cells, these results confirmed the potential use of $C_{specific\ membrane}$ and $\sigma_{cytoplasm}$ for tumor cell classification, and paved the way for future clinical applications.

2. Materials and methods

2.1. Materials and cell culture

Unless otherwise indicated, all cell-culture reagents were purchased from Life Technologies Corporation (Van Allen Way Carlsbad, CA, USA). Materials required for device fabrication included SU-8 photoresist (MicroChem Corp., Newton, MA, USA) and 184 silicone elastomer (Dow Corning Corporation, Midland, MI, USA).

Non-small-cell lung cancer cell lines 95D, 95C, A549 and H1299 were cultured at 37 °C in 5% CO_2 in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin, respectively.

2.2. Oncogene regulation

Small interfering RNA-mediated gene regulation was used to down regulate two oncogenes: *Cyclin Y (CCNY)* (Yue et al., 2010; Yue et al., 2011) and *Cyclophilin A (CypA)* (Li et al., 2006; Obchoei et al., 2009; Qian et al., 2012; Yang et al., 2007), respectively. Four RNAi candidate target sequences to CCNY were designed and cloned into the recombinant vector (Shanghai GeneChem Co. Ltd, Shanghai, China). CCNY-Si1 (AAATGTGTCGCTCTTGCAATA), which corresponds to nucleotides 523–543, was the most effective at decreasing CCNY mRNA levels in 95D cells and used to knockdown the endogenous CCNY in the following experiments.

Four CypA-targeting oligonucleotides serving as RNAi candidates were designed based on the full-length CypA cDNA sequence and cloned into the recombinant vector (Shanghai Genechem Co. Ltd., Shanghai, China). CypA-Si2 (CTGACTGTGGACAACTCGAAT), which matches the sequence located at nucleotides 559–579 of the CypA cDNA, was proved to be the most effective at decreasing the CypA mRNA level in A549 cells and used to knockdown endogenous CypA in the following experiments.

2.3. Transwell assay and western blot

To characterize the migration capabilities of paired high-metastatic and low-metastatic carcinoma strains 95D and 95C cells (He et al., 2001; Su et al., 2005; Sun et al., 2012; Wang et al., 2013), the Boyden chamber assay with an 8 μm barrier membrane was used. The upper chamber was filled with 8×10^5 cells/mL with culture medium plus 0.2% bovine serum albumin (a total volume of 100 μL) while the lower chamber was filled with culture medium supplemented with 10% fetal bovine serum as the chemo-attractant (a total volume of 600 μL). After 8 h of cell culture, the cells on the upper surface were removed by gentle abrasion with a cotton bud, and the cells on the underside (migrated cells) were fixed and stained. The mean number of cells on the lower side of the surface was counted from five randomly chosen high-power fields (× 40) under light microscopy in three independent experiments.

Western blot was used to quantify expressions of the oncogenes under regulation. Cells (95D cells, 95D CCNY-KD cells, A549 cells and A549 CypA-KD cells) were homogenized in the ice-cold lysis buffer (10 mM Tris–HCl, pH 7.4, 1 mM EDTA, 0.1% Tritron X-100, 0.1% SDS) containing protease inhibitors (2 μg/mL aprotinin; 10 μg/mL antipain, 2 μg/mL pepstatin, and 2 mM benzamide). Cell lysates were centrifuged (10,000 g for 10 min at 4 °C) and the resulting supernatant mixed with the loading buffer was separated by the SDS-PAGE gel and wet-transferred into PVDF membranes for 2 h followed by immunoblotting. The antibodies against CCNY and CypA were used at a dilution of 1:1000 and 1:500, respectively, and incubation was carried out overnight at 4 °C. Secondary antibodies conjugated with horseradish peroxidase (1:10,000 for CCNY and 1: 5000 for CypA) were incubated with PVDF membranes for 1 h at room temperature.

2.4. Device fabrication, operation and data analysis

The two-layer PDMS device (constriction channel cross-section area of 10 μm × 10 μm or 10 μm × 12 μm) was replicated from a