MICROSCALE CULTURE AND ENRICHMENT OF CANCER STEM CELLS FOR DRUG DEVELOPMENT

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ABSTRACT

Screen for drugs that specifically kill epithelial cancer stem cells (CSCs) has not yet been realized due to the rarity of these cells within tumor cell population and their instability in culture [1]. Here we present a microfluidic chip that can facilitate culture and enrichment of cells with CSC-like properties *in vitro*. Furthermore, we show that human epithelial cancer cells can grow to a tumor spheroid, acquire properties of mesenchymal cells (an increased ability of migration), and have a gain of CSC properties (an increased drug resistance). In addition, this chip requires no external pumps and tubings yet being pipette-friendly in usage.

KEYWORDS

Cancer stem cell, microfluidic, spheroid, drug resistance, pipette-friendly, EMT

INTRODUCTION

A direct link between epithelial-mesenchymal transition (EMT) and the gain of epithelial stem cell properties, anincreased ability to form mammospheres, has been demonstrated [2]. In addition, to create the cellular micro-environment and recapitulate the physiological conditions, a chip capable of generating precisely micro-scale patterning of cells is crucial. Based on these, we propose patterning of epithelial cancer cells in a microchip, with particular feature of cell low-attachment as such heterologous tumor spheroid will form cells as they undergo EMT, acquire an increased metastatic ability, and have properties of stem cells.

EXPERIMENT

Figure 1 shows the schematics of the microfluidic chip and procedures of drug screening on cancer stem-like cells induced from the microscale culture. This chip includes an engineered PDMS membrane, top and bottom channels, and four open wells for introducing agents by manually pipetting as described [3].

RESULTS AND DISCUSSION

Figure 2 shows 3D culture on chip can generates cells with enhanced EMT properties, in which cells from the microchip acquired fibroblast-like appearances [Figure 2(a)] and had a lower expression of epithelial marker (CD326/EpCAM) and a higher expression of mesenchymal marker (vimentin) to those in traditional

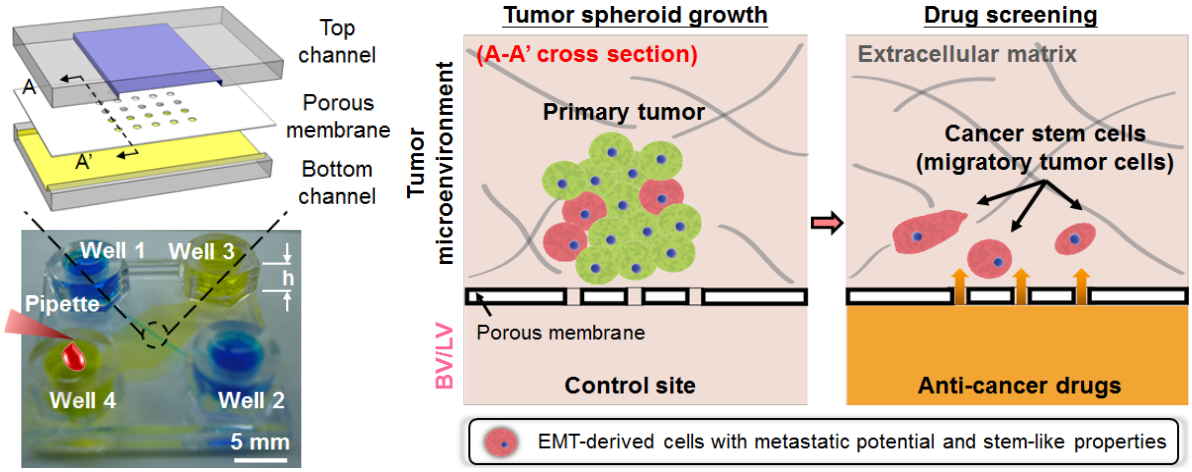

Figure 1: Schematics showing (a) the microfluidic chip design, including a 3D perspective view of the cell handling area and photograph of the fabricated chip, and (b) procedures for drug screening on cancer stem-like cells from a primary tumor, in which A-A' cross section is from (a). BL/VL, blood vessels/lymphatic vessels. Cells used in this study were human ovarian cancer cells, SKOV3.

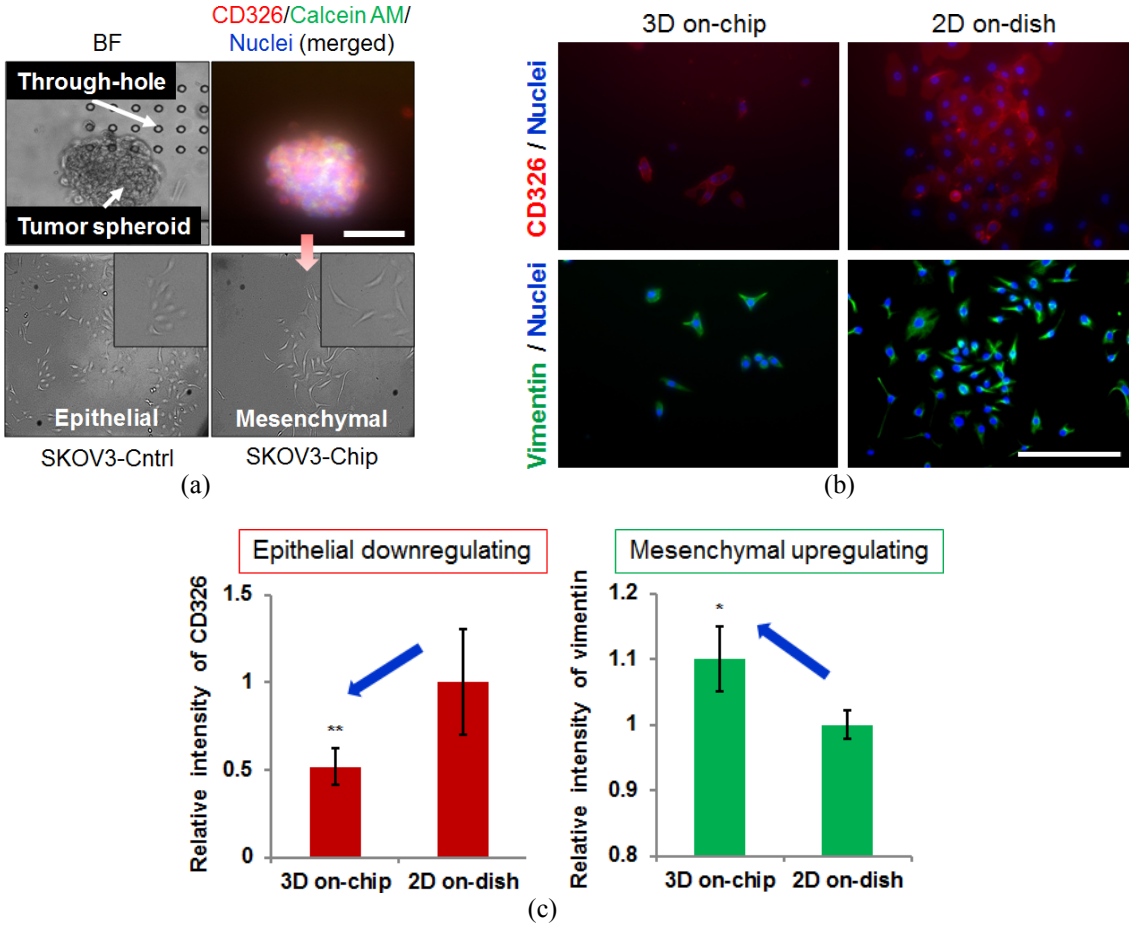

Figure 2. 3D culture on chip generates cells with enhanced EMT traits. (a) Differential cell morphology on cells between on chip and traditional 2D cultures (SKOV3-Cntrl). (b) Immunofluorescence detection of CD326/EpCAM (red), vimentin (green), and Hoechst nuclear staining (blue) with cell monolayers from (a). (c) Total area signal intensities in the cell monolayers. Data represent the mean ± SD, n=5, (-p<0.05, **-p<0.01). Scale bars, 100 μm.*

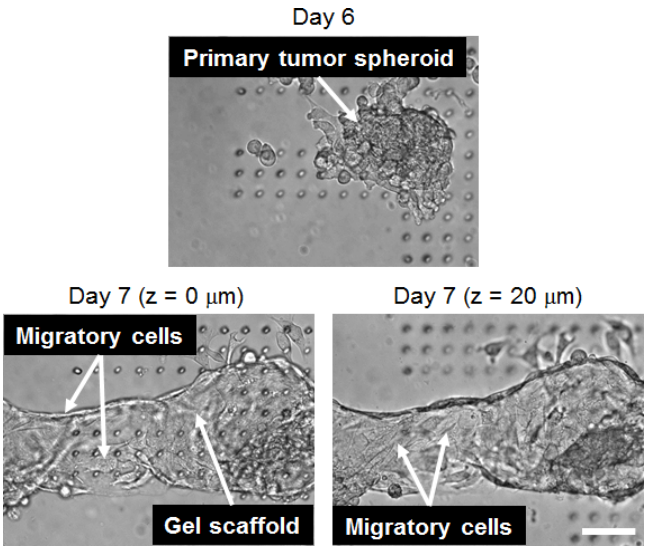

Figure 3. Migration of tumor cells in 3D in the microfluidic chip. Results show some cells migrated to a distant site (~200 μm far) from the primary spheroid at day 7. Scale bar, 100 μm.

2D cultures [Figures 2(b) and (c)]. Metastasis causes most cancer deaths, yet this process remains one of the most enigmatic aspects of the disease. In this work, we have also demonstrated this microchip has the feasibility of recapitulating the migration of tumor cells in 3D matrices *in vitro* (Figure 3), which may enable the modeling of complex metastatic cascade in the near future.

Drug treatment of cancer cell populations leads to concomitant enrichment for CSCs and for cells that have undergone an EMT [1]. We therefore examined whether ovarian cancer cell populations that have been

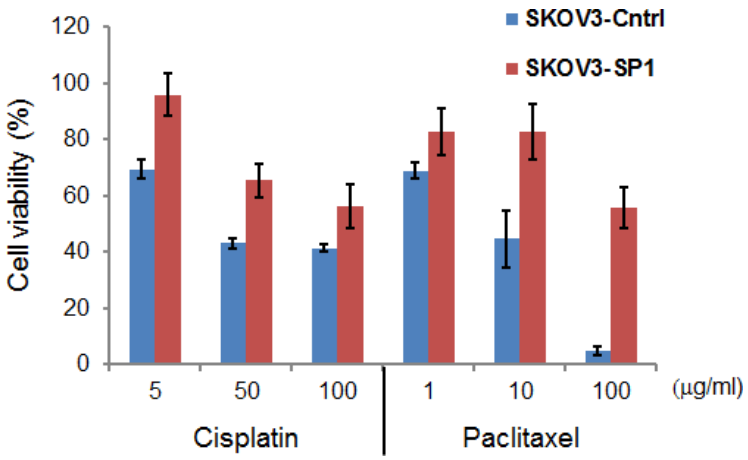

Figure 4. Viability of native ovarian epithelial cells (SKOV3-Cntrl) and cells induced through EMT (SKOV3-SP1) treated with two chemotherapy compounds. Data represent the mean ± SEM, n=3.

experimentally induced into EMT also share this aspect of CSC biology, namely an increased resistance to chemotherapeutic drugs. We found that SKOV3-SP1 cells were more resistant than SKOV3-Cntrl cells (parental cell lines) to two commonly used chemotherapeutic drugs, cisplatin (~20-fold increase in IC_{50}) and paclitaxel (~40-fold increase) (Figure 4).

CONCLUSION

In conclusion, we interrogated a microfluidic chip that can facilitate tumor spheroid cultures and drug screens. Furthermore, we demonstrated this microchip can enhance EMT properties from cultured spheroids and has a potential for the study of cancer metastatic cascade. In other words, this microchip may achieve a potential long-term goal for the possibility of accomplishing "organotypic cultures" that represent *in vitro* tumor microenvironment for personalized drug screening.

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