THE SIGNIFICANCE OF NUCLEAR DEFORMATION FOR CANCER CELL TRANSMIGRATION

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ABSTRACT

In this paper, we present a microfluidic platform for the study of cancer cell transmigration and anti-cancer drug screening. The device consists of an array of microchannels with dimensions close to the capillaries on the cellular barriers, while a thin glass coverslip is used as the substrate to enable high resolution imaging of cell dynamics during cancer cell transmigration. We have investigated the transmigration of highly metastatic human breast cancer cells, MDA-MB-231, after their cell nucleus are fluorescently labeled. The results show that 1) the cell nucleus deformed drastically during the transmigration process, and 2) an anti-cancer drug targeting the cell nucleus significantly inhibited transmigration.

KEYWORDS

Cancer transmigration, nucleus, deformation, anticancer drug

INTRODUCTION

Transmigration of cancer cells is a critical step in the process of metastasis. In order to move across the narrow openings found in cellular barriers that separate the organs from the circulation, cancer cells need to undergo significant physical deformations as shown in Fig. 1. Consequently, factors governing their stiffness and deformability are critical to their transmigration efficacy and metastasis potential. [1-4]

The nucleus is the largest and stiffest cellular organelle, which consists of dense genetic materials surrounded by lipid bilayer envelop with underlying networks of structural proteins. [5] This high stiffness usually makes nuclear deformation the critical step during transmigration. Therefore, pharmacological or biological approaches that inhibit nuclear deformation might prevent the transmigration of cancer cells and subsequently reduce their metastatic capabilities.

Studies on cancer cell transmigration have been hampered by a shortage of suitable platforms. In this paper we present a novel microfluidic platform which allows live monitoring of nucleus dynamics. It can also be used to evaluate the effect of anti-cancer drug on the prevention of transmigration under different degree of spatial restrictions.

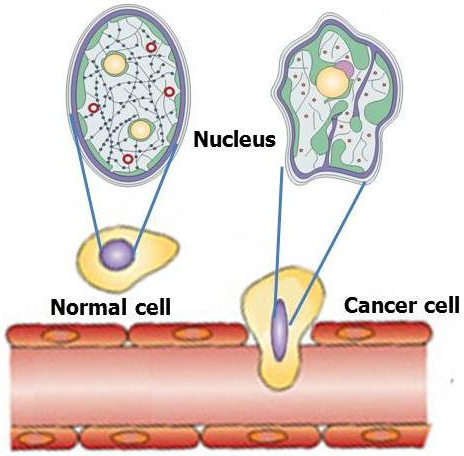

Figure 1: Nucleus deformation and cancer cell transmigration. Cancer cells have altered nuclear inner structure, which affect their stiffness and may facilitate their transmigration during metastasis.

CHIP DESIGN AND FABRICATION PROCESS

Figure 2 shows a schematic illustration of the microfluidic transmigration device. It consists of a center chamber for seeding and the cancer cells and two chemoattraction chambers linked to the culture chamber by arrays of microchannels with a height of 5μm, a length of 100 μm and widths of 3, 6, 9 and 12 μm respectively. The dimensions of the microchannels are designed to be close to the capillaries on the cellular barriers. When chemoattractants (present in fetal bovine serum) are applied to the side chambers, concentration gradients are established along the microchannels to attract the cancer cells and stimulate their migration. A center ridge is designed in the middle of the chemo-attraction chamber to prevent mixing of cells that migrated through different microchannel widths. The entire device is mounted on top of a glass coverslip for high quality imaging of the cellular components.

A two-step photolithography method was used in the fabrication process. [6-8] First, a thin layer (5 μm) of SU-8 10 photoresist was spin-coated on a wafer and exposed with the first chrome mask, which defined the microchannel widths (3–12 μm). Then a second thick layer (100 μm) of SU-8 100 photoresist was spin-coated on the first layer and exposed with a second mask to make the chambers for cell culture and chemo-attraction. Polydimethylsiloxane (PDMS) prepolymer and curing agent (10:1) was poured over the master, degassed and baked 2 hours at 75 ℃ and then peeled off. The inlets and outlets holes were punched manually. Then, the PDMS devices were exposed to air plasma for 15 s in order to bond it with

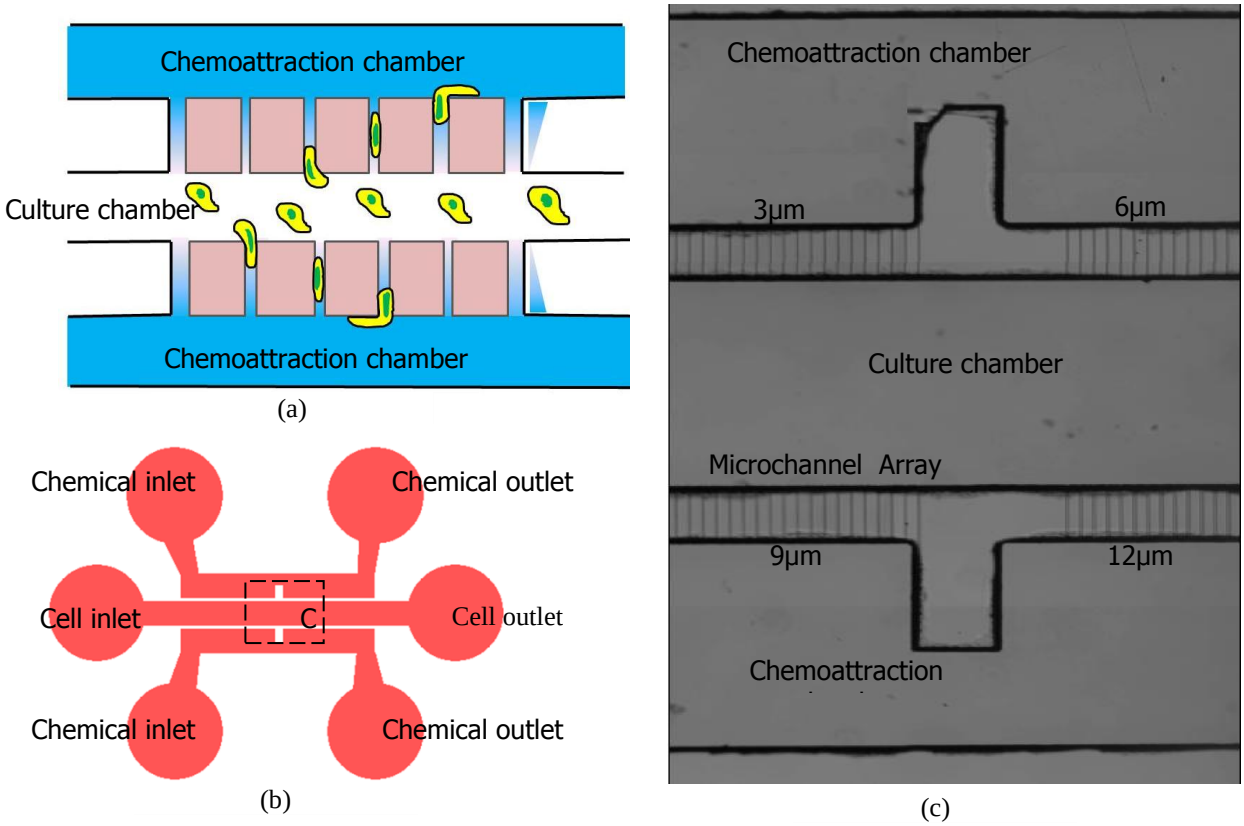

Figure 2: Schematic illustration of (a) microfluidic device for the study of cancer cell transmigration, (b) top view of chamber design, and (c) zoom-in view of fabricated microchip showing different widths of restriction channels.

the glass coverslip.

EXPERIMENTAL RESULTS AND DISCUSSIONS

Transmigration of highly metastatic MDA-MB-231 cells was studied. Fig. 3(a) shows an overview of the transmigration of MDA-MB-231 cells in the microfluidic device. After serum starvation in the cell culture chamber and application of FBS

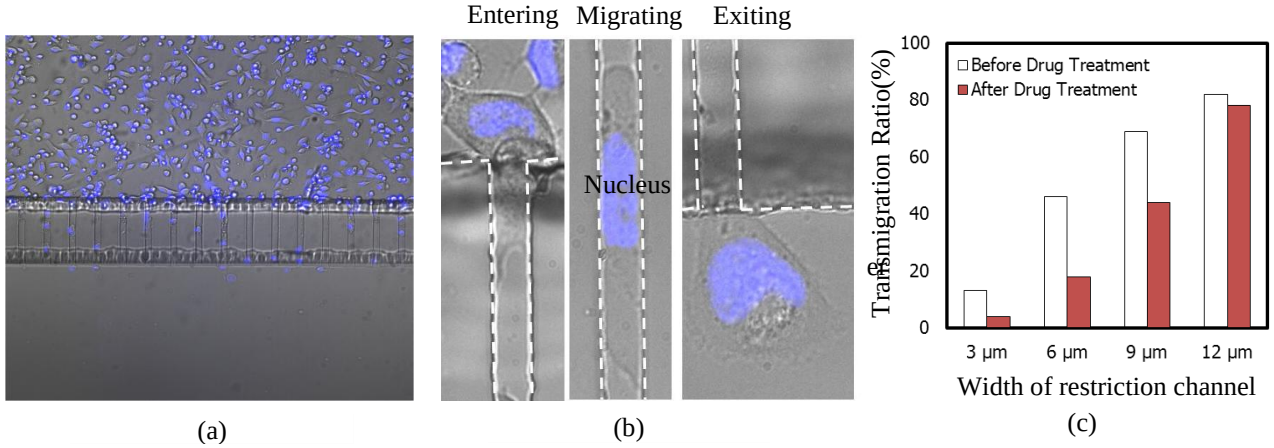

Figure 3: Migration of MDA-MB-231 cells in the microfluidic transmigration device. Walls of microchannels are highlighted by white dot lines. Nucleus of the cell was stained with fluorescent dye Hoechst 33423. (a) Overview of cells migrating through microchannels (b) Zoom-in view of cells at different stage of transmigration.(c) Effect of anti-cancer drug on the transmigration profile of MDA-MB-231 cells in different widths of restriction channels.

to the chemoattractant chambers, a concentration gradients of the chemokines in FBS was established along the microchannels and provided cues for cell transmigration. This is a general behavior of the cells commonly known as chemotaxis. Details of the transmigration process were shown in Fig. 3(b). Transmigration occurred in several distinct steps. First, cells came into contact with the entrance of the channel and extended their cytoplasmic portion into it. During this process, the cytoplasm quickly adjusted its shape based on the geometry of the microchannel. Because of the relatively high stiffness of the nucleus, cells got stuck at the entrance. The nucleus deformed from a spherical to an elongated ellipsoidal shape required for transmigration across the microchannel. The shape of the nucleus restored to spherical after the cell exited the microchannel. Statistical analysis showed that the average nuclear size increased significantly after the cells transmigrated across the microchannel, which might be related to an alteration of nucleus membrane and intra-nucleus components.

Figure 3(c) shows the effect of an anti-cancer drug with an ability to change nuclear inner structure on the overall transmigration profile of MDA-MB-231 cells in the microchannel array. At each channel dimension, a significant decrease in the percentage of the cells to be able to complete the transmigration was observed after drug treatment. The effect was more obvious in the small channels (width < 9 μm) as it required more drastic deformation of the nucleus. The percentage dropped from 46% to 18% in 6 μm channel and from 13% to 4% in 3 μm channel.

CONCLUSIONS

In conclusion, nuclear deformation during transmigration of breast cancer cells was studied using a microfluidic transmigration platform. The results show that the cell nucleus deformed drastically during the process and anti-cancer drug targeting the cell nucleus significantly prevent their transmigration. Since transmigration is critical for cancer metastasis, the results are useful in designing new anti-cancer therapy.

ACKNOWLEDGEMENT

This work is supported by the Science & Engineering Research Council of Singapore (Research project with Grant No. SERC 102 152 0013 and 102 171 0159).

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