A STUDY OF CANCER CELL METASTASIS USING MICROFLUIDIC TRANSMIGRATION DEVICE
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ABSTRACT
Metastasis of cancer cell was studied using microfluidic device. Migration of cells in the microchannel array mimics their transmigration in tissue capillaries during in vivo metastasis. Details of the morphological changes were recorded through high resolution imaging. Results showed that the deformation of cell nucleus is critical for the transmigration of breast cancer cell MDA-MB-231. Condensation of chromatin by drug plicamycin significantly impaired their deformation abilities and subsequently reduced the transmigration by 2 to 3 fold for microchannel smaller than 10 μm × 5 μm. As transmigration is critical for cancer metastasis, the results will be useful in designing new anti-cancer therapy.

INTRODUCTION
The transmigration of cancer cells across the cellular barriers are critical steps during their metastasis. For them to trespass the narrow openings on the barriers, which might be smaller than their original sizes, cancer cells need to undergo lots of physical deformations during the process. Therefore, factors affecting their stiffness and deformability are critical to their transmigration and metastasis [1-2].

The cell nucleus consists of highly condensed chromatins and organized networks of structural proteins. The compact structure grants the nucleus with high mechanical strength [3]. Hypothesis has long existed that nuclear deformation is the primary rate-limiting step during cell transmigration [4]. Cancer cells usually have disrupted chromatin arrangement and altered nuclear morphologies as compared to normal cells [5]. Biologists have argued that these changes result in the decrease of nucleus stiffness and facilitate the transmigration of cancer cells during metastasis (Fig. 1) [6-7].

Proofing these hypotheses is difficult to be achieved on conventional platform such as Boyden chamber or other membrane-based devices. They are relatively bulky and the migration events occur too far from the surface for cell imaging. And usually each chamber consists of pores of the same diameter thus study the effect of pore dimension using the same experimental conditions on a single device is impossible [8]. In recent years, many novel devices have been created for the transmigration studies using microfluidic technology [9-11]. With advantages such as precise control of microfluidic environmental parameters and high-resolution imaging, these new platforms have provided valuable insights in the transmigration studies.

In this paper, the role of nuclear deformation in the transmigration of breast cancer cell MDA-MB-231 is studied using microfluidic device. After its importance is manifested, chromatin-condensing drug is applied and their effects on the prevention of cancer cells’ transmigration are studied.

DESIGN AND FABRICATION OF MICROFLUIDIC DEVICE
Schematic illustration of the microfluidic transmigration device is shown in Fig. 2. It consists of two chambers linked by an array of microchannels with height of 5 μm, length of 100 μm and width ranging from 6 μm to 15 μm. Cells are grown in the upper culture chamber. When chemoattractants is applied to the bottom chamber, a concentration gradient is established along the microchannels to attract the cancer cells to migrate through. This process mimics the transmigration of cancer cells in tissue capillaries during metastasis. Drugs can be applied directly to both chambers through inlets.

The microfluidic device is fabricated using standard soft-lithography techniques [12-15]. A two-step photolithography method was used to in the fabrication process. First, thin layer (5 µm) of SU-8 10 photoresist (MicroChem) was spin-coated on wafer (CEE 200, Brewer Science). After soft baking, it was exposed with the first chrome mask which defined the microchannel widths (6–15 μm) followed by the post
exposure bake. 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 chemoattraction. Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) prepolymer and curing agent (10:1) was poured over the master, degassed and baked 2 hours at 75 °C and then peeled off. The inlets and outlets holes were punched manually using a Harris uni-core sampling puncher (5 mm). Then, the PDMS devices were exposed to air plasma for 15 s using a corona treater (BD-25, Electro-Technic Products) to bond it with glass coverslip.

**EXPERIMENTAL RESULTS AND DISCUSSIONS**

All reagents were purchased from Invitrogen. The device was pretreated with bovine fibronectin solution to facilitate cell attachment on the glass coverslip. Human breast cancer cells (MDA-MB-231) were maintained in minimal essential media (DMEM). Adherent cells were detached using trypsin, and resuspended in low serum medium at a density of $1 \times 10^7$ cells mL$^{-1}$. The nucleus of the cell was stained with fluorescent dye, Hoechst 33342, prior to cell loading into the chamber. Cells were injected into the inlet of the culture chamber and incubated at 37°C and 5% CO$_2$ for 1 hour to allow the cells to attach and spread. DMEM supplemented with chemoattractant (20% fetal bovin serum (FBS)) was then injected into the inlet of chemoattraction chamber. The device was then placed on an Olympus IX81 microscope equipped with a temperature controller and a CO$_2$ incubation chamber. For drug experiments, drugs was diluted in the medium with a concentration of 10 µM and applied to the culture chamber after cell attachment. The device was then incubated at 37°C and 5% CO$_2$ for 16 hour before loading the chemoattractant.

Figure 3 shows the overview of the migration of MDA-MB-231 cells. After the application of chemoattractant for 12 hours, 58% of the cells migrated cross the microchannels to the chemoattractant side. In addition to the attraction force provided by the concentration gradient of the chemoattractant, the cells need to deform themselves in order to squeeze through
the microchannels with small dimension of cross-sections. Figure 4 shows the transmigration process of a cancer cell through a microchannel with cross-sections of $9 \mu m \times 5 \mu m$ (W×H). The transmigration occurred in several distinct steps. First, cells came into contact with the entrance of the channel. Then, they identified the pore by extending the cytoplasm portion into it and migrate toward the pore opening. During this process, the cells quickly adjusted their shapes according to the geometry of the microchannel. It took 15 minutes for the entire cytoplasm portion of the cell to enter the microchannel before the nucleus of the cell falls behind and stuck at the entrance. It took relatively longer time (80 minutes) for the nucleus to deform and squeeze in. After the entire cells have entered the microchannel, cells migrated at a speed approximately 35 $\mu m/hour$ toward the chemotraction chamber.

As the results showed that the deformation of the nucleus is primary rate-limiting step in MDA-MB-231 cell transmigration, the nuclear deformability is reduced using chromatin-condensing drug, plicamycinon, to investigate its transmigration. Effect of plicamycinon on the cells is shown in Fig. 5. After treating the cells with 10 $\mu M$ plicamycinon for 16 hour, chromatin condensation was observed, which resulted in the reduction of nuclear size and the change of morphology from irregular shape to tight oval shape.

Figure 6 shows the migrating velocity of the cells in the microchannels before and after drug treatment. In small channels (width < 10 $\mu m$), drug treated cells migrated significantly slower than non-treated cells, average velocity dropped from 44 $\mu m/hour$ to 29 $\mu m/hour$ in 9-$\mu m$ channel and from 28 $\mu m/hour$ to 17 $\mu m/hour$ in 6-$\mu m$ channel. Fig. 7 shows the effect of the drug on the overall transmigration profile of MDA-MB-231 cells in the microchannel array. At each channel dimension, significant reduced number of the total percentage of the cells able to complete the transmigration could be observed after drug treatment. The effect was more obvious in small channel (width < 10 $\mu m$) as it required more drastic deformation of the nucleus. The percentage dropped from 46% to 18% in 9-$\mu m$ channel and from 13% to 4% in 6 $\mu m$ channel.
REFERENCES

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