Quantitative Comparison of Metastasizing and Non-metastasizing Breast Cancer Cell Migration via Various Dimension Microchannels

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Introduction

As many as 90% of cancer related deaths are due to the metastasis of primary tumor cells. With breast cancer being the second most diagnosed cancer as well as the leading cause of death in women, an understanding of the differences between metastasizing and non-metastasizing breast cancer is critical. We explored the outcome of metastasizing (MB-231) and non-metastasizing (MCF-7) breast cancer being cultured on collagen, the most abundant protein in the extracellular matrix of the breast tissue. We also conducted studies on the migratory patterns of MB-231 and MCF-7 through different sized microchannels (3, 5, 8, 10, 15 and 20µm). Finally, analysis of the drug Paclitaxel (Taxol) was done where the effect of the drug on breast cancer cells was quantified for various dimension microchannels and drug concentrations (0-20µM). For all of the experiments, Polydimethylsiloxane (PDMS)-based microchannel devices were used. For the migration study, the device used had a cell seeding reservoir connected to six surrounding satellite reservoirs by different width microchannels (Flower microchannel device). For the drug study, the device had one cell seeding side connected to one reservoir by microchannels of either 5 by 5µm or 15 by 15µm (width by height). The purpose of using those devices was so that we could control the polarity of the migrating cells and follow specific cells throughout their migration process.

Materials and Methods

Breast cancer cell culture: Prior to the cells being seeded, the devices were punched, washed and decontaminated, then attached to a glass slide. A collagen coating was applied to the devices and incubated overnight. After washing the devices three times with PBS solution, the cells were seeded and closely monitored for data collection and analysis.

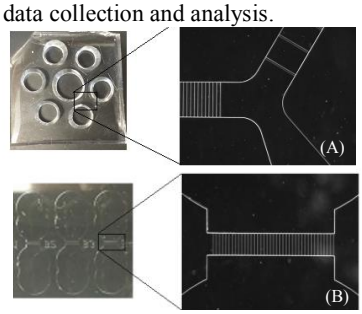

Figure 1: (A) Device used for cell migration study. Six satellite reservoirs connect to a central reservoir by different width microchannels (Flower device). (B) Device used for drug study. The cell seeding reservoir (top) is connected to the bottom reservoir via microchannels

Migration study of breast cancer cells: Before the cells were seeded, 60µl of media was removed from the center reservoir, and then 50,000cells/device in 50µl was seeded directly in the center of the central reservoir. These methods were used to avoid seeding bias, resulting in even distribution of the cells toward all six types of microchannels. The numbers of cells inside the channels and those that had entered and exited on the other side were counted.

Effect of anti-cancer drug Taxol on breast cancer cell migration via various physical confinements: Media was placed into the reservoirs and 20,000cells/device were shot towards the channels. Because there was only one size channel connecting the two reservoirs, seeding bias was not a concern. After two days, the cells had entered the microchannels (either 5µm by 5µm, narrow channel or 15µm by 15µm, wide channel) and different concentrations of the drug were introduced. Three hours later, pictures of the cells in the channels were taken. Five hours after that, pictures were taken again. Using Image J software, we quantified the speed of the cells, migrating via either narrow or wide microchannels.

Results and Discussion

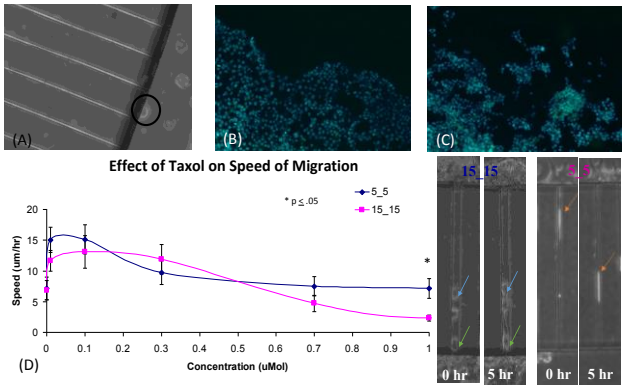

Figure 2. (A) MB-231 in a satellite reservoir after crossing a 3µm channel. (B) MB-231 migrating in an epithelial sheet on glass without collagen coating. (C) MB-231 migrating as a single unit on collagen coated glass. (D) The effect of different concentrations of Taxol on metastasizing breast cancer (Left). Representative pictures of cells in a 15µm by 15µm channel (Middle) and a 5µm by 5µm channel (Right). n=20/each dose. *P<0.05

Both metastasizing and non-metastasizing cells were able to travel through channel widths as small as 3µm (Fig. 2A). Using a collagen coating on the devices provided us with a unique difference between MB-231 and MCF-7. It was found that when seeded on collagen or glass, MCF-7 moved in an epithelial sheet, but metastasizing MB-231 cells moved as a single cell when on a collagen coating (Fig.2B and C). Quantitative analysis showed that metastasizing breast cancer enters and exits the microchannels more quickly than non-metastasizing. After five days, metastasizing cancer had entered and exited all six sizes of channels, whereas non-metastasizing had entered only the four larger channels and had not exited any of them. This demonstrates not only that the metastasizing cells have the ability to migrate faster in general, but that they are also able to move through confined spaces faster. Due to the slow migration of MCF-7, only MB-231 cells were used in the drug study. This experiment showed that the migration speed is 2 times reduced at 1µM Taxol in confined channels, but it is 4 times reduced in wide channels (Fig. 2D). This result indicates that Taxol is significantly less effective in the confined microchannel, which is similar to a tightly packed tissue, than in the wide microchannels, which is similar to a 2D environment like a Petri dish. This raises the question of the impact that Taxol actually has on vigorously migrating (metastasizing) cancer cell in the human tissue.