

LYMPHATIC CAPILLARY INVASION ASSAY BY A SINGLE CELL MIGRATION CHIP

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ABSTRACT

We demonstrated a single cell migration chip which can emulate cancer cell invasion in lymphatic capillaries through a migration channel with resistance choke points. Using a hydrodynamic capturing scheme based on the difference in flow resistance, the device allows for positioning single cells at the start of a migration channel. Different sizes of the resistance choke points in the migration channels are investigated to characterize the deformation capability of cells. To verify the migration platform, we used p38 γ gene knockdown MDA-MB-231 (breast cancer) cells, which are known to show lower lymphatic metastasis *in-vivo* in a separate study. The reduction of the invasive ability in lymphatic capillaries was confirmed by the fabricated migration chip.

KEYWORDS: Lymphatic Capillary Invasion, Single Cell, Cell Migration, Metastasis

INTRODUCTION

Cell migration is an essential process in angiogenesis, cancer metastasis, wound healing, inflammation, and embryogenesis. Significant attention has been paid to the migration of cancer cells since cancer metastases account for more than 90% of cancer-related mortality [1]. Several metastasis-suppressor genes, which may be potential targets for therapeutics, have been discovered and are actively investigated [2]. Nevertheless, the study of metastasis-related genes still mostly depends on xenograft models, which require a great amount of time and cost and are difficult to adapt to personalized therapy in real time [3]. Hence, there is an unmet need to develop *in-vitro* devices which can emulate metastasis, especially for the lymph node invasion process which is a critical step in the metastasis of cancer through the lymphatic capillaries, and devices that can also be potentially employed for therapeutic decision making in the clinic [4].

p38 γ mRNA is overexpressed in several types of cancer and helps increase Ras-induced cancer invasion. Knockdown of p38 γ genes degrades the efficiency of lymph invasion *in-vivo* partly due to drastically changing the type of motion the cell can undergo [5]. It is believed that p38 γ knockdown leads to ubiquitination and degradation of RhoC [5]. Due to a lack of RhoC, the knockdown cells (GKD) are unable to form long pseudopodia and thus engage in motility that does not effectively lead to cellular displacement. The resulting unorganized cytoskeleton reduces the efficiency of cell movement. To characterize the invasion capability of MDA-MB-231 (breast cancer) cells in a 3D model of lymphatic capillaries, we devised a single cell migration chip that contains multiple migration resistance choke points and successfully demonstrated the capability of tracing single cells in this lymphatic capillary invasion assay.

DESIGN AND FABRICATION

Figure 1 shows the schematic diagram of the devised chip. Cells are loaded by gravity flow and hydrodynamically captured on the left-hand side of the migration channels; chemoattractants are added to the right-hand side, and the concentration gradient is generated by diffusion [6]. Cell migration in the channel is observed after 24 hours. PDMS (polydimethylsiloxane) layers were fabricated on a glass substrate by the standard fabrication process. Three masks are used to fabricate the multiple heights for the channel region (40 μm height), the capture gap (20 μm height), and the migration channel (10 μm height). Figure 2 shows the fabricated device and four different designs of the migration resistance choke points (100 μm length) for emulating the geometry of lymphatic or other capillaries [7]. The width of the choke points varies from 6 μm to 30 μm . The motility was scored by the number of choke points passed by cells as indicated in the upper part of Figure 2.

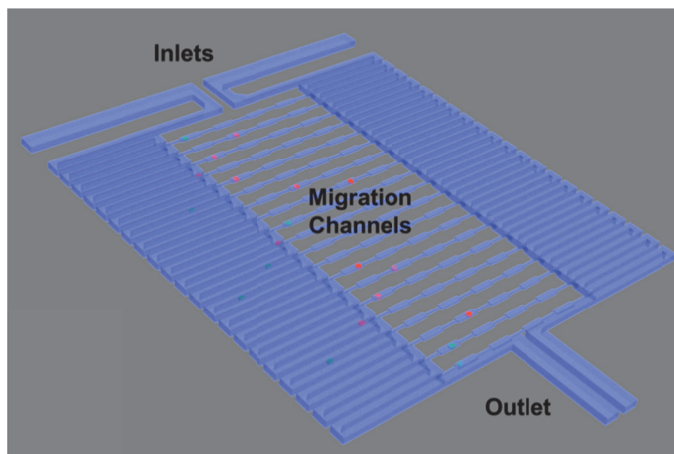


Figure 1. Schematic diagram of the single cell migration chip with invasion resistance choke points

EXPERIMENTAL

MDA-MB-231 cells were cultured in RPMI with 10% FBS and 1% penicillin/streptomycin. p38 γ knockdown MDA-MB-231 (GKD) cells were stably transfected with short hairpin RNA (shRNA), and the scrambled vector (SCR) cells were the standard cell line (Merajver lab, [5]).

Before cell loading, trypsin/EDTA is used to re-suspend the cells in solution, which are diluted to 10^6 cells/mL and pipetted into the inlet. Liquid height difference between the inlets and outlets generates gravity flow, and thus cells are captured hydrodynamically. After 10 minutes, the cell solution in the inlet is replaced by serum-free culture media, and 20% Fetal Bovine Serum (FBS) media is applied to the other inlet to induce migration. Then, the entire chip is put into an incubator. Migration behavior is observed after 24 hours, and the velocity of cells is measured by monitoring every 30 minutes.

RESULTS AND DISCUSSION

First, we observed cell migration behavior in the 6 μ m resistance choke point migration channels. Figure 3 shows the representative morphologies of scrambled vector (SCR) cells and p38 γ knockdown (GKD) cells. F-actin fibers are labeled by red fluorescence. Since SCR cells were able to form long pseudopodia past the choke point, the cells were elongated and migrated through the narrow channel by contraction of stress fibers. Due to the lack of RhoC and other global effects of p38 γ knockdown, GKD cells could only squeeze into the narrow channel.

Next, we observed cell migration distance for various chemoattractants without resistance choke points, as shown in Figure 4. Both 50 ng/mL hepatocyte growth factor (HGF) and 20% FBS induced migration. After confirming chemotaxis of MDA-MB-231 cells, we tested the dependence of cell motility on the dimensions of migration choke points (Figure 5). In this experiment, we used 20% FBS media as the chemoattractant since HGF may also regulate RhoC and confound the results [8]. We observed that SCR and GKD cells have a similar motility when the migration channel is wide (30 μ m x 10 μ m) and without choke points, but the motility of GKD cells significantly diminishes when the channel is obstructed by narrow choke points (6 μ m x 10 μ m).

To verify the lower migration efficiency of p38 γ knockdown cells, the velocity of MDA-MB-231 cells in the narrowest choke point channel (6 μ m x 10 μ m) is measured as shown in Figure 6. Although the variation is large, the velocity of SCR cells is almost double that of GKD cells. This result confirms the hypothesis that the motility of GKD cells decreases due to a lack of RhoC. The actual distribution of cells for various choke

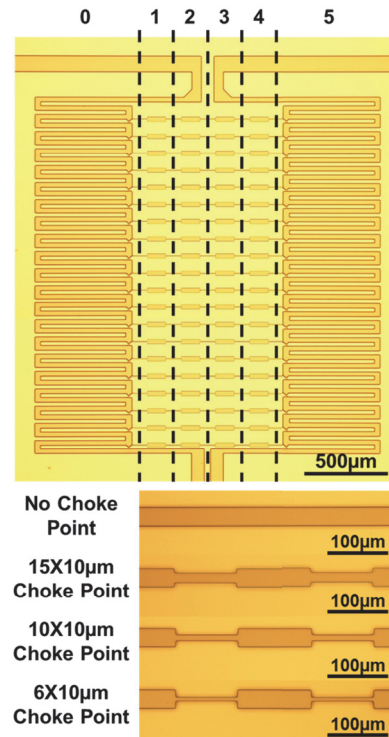


Figure 2. Microphotograph of the fabricated device and size variation of migration channels.

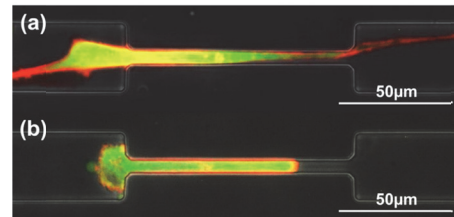


Figure 3. MDA-MB-231 cells (F-actin is labeled by RFP) in the 6 μ m x 10 μ m choke points : (a) scrambled (SCR) cell and (b) p38 γ knockdown (GKD) Cell.

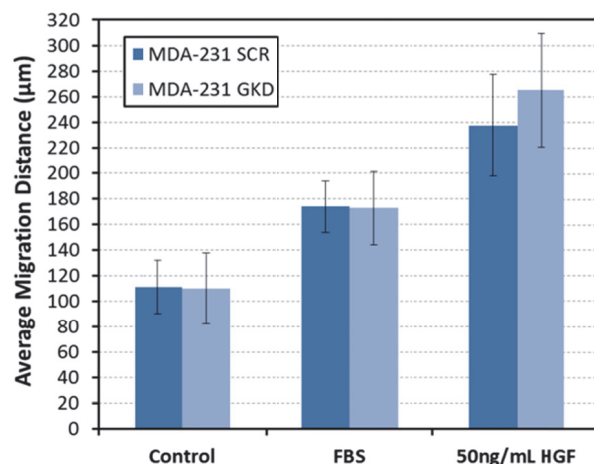


Figure 4. Migration distance of MDA-MB-231 cells for different chemoattractants without choke points.

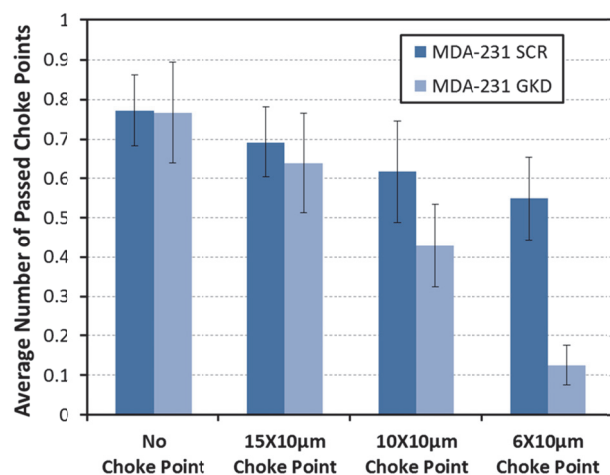


Figure 5. Average number of choke points passed by SCR and GKD cells for various choke point dimensions with 20% FBS as the chemoattractant.

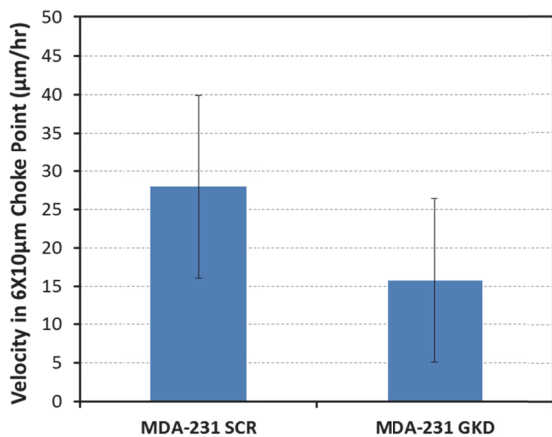


Figure 6. Average velocity of MDA-MB-231 cells in the migration channel with choke points of $6\ \mu\text{m} \times 10\ \mu\text{m}$.

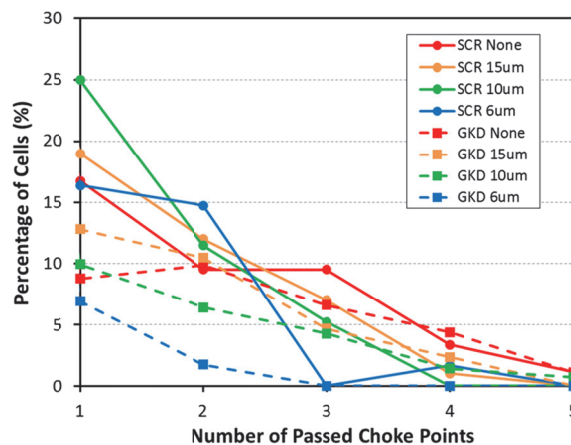


Figure 7. Distribution of cells (SCR and GKD MDA-MB-231 cells) as a function of passed resistance choke points for various dimensions.

point dimensions is depicted in Figure 7. Most cells passed fewer than two choke points and very few cells passed all five choke points. The heterogeneous behavior of cells in the channel can also be studied in future work.

CONCLUSION

In this work, we implemented a single cell migration platform with choke points to emulate cancer cell invasion in the lymphatic capillaries by adjusting the choke point dimensions. Using a hydrodynamic capture scheme, single cells can be placed at the start of a migration channel. The novel design of resistance choke points allows us to study the migration behavior of cancer cells in a 3D model. As a proof of concept, p38 γ gene knockdown MDA-MB-231 cells were tested to show that they had significantly reduced motility as compared to scrambled cells when migrating in the narrowest choke point channel. Due to the nature of single cell resolution, heterogeneous cell populations can be labeled and traced in the chip, and the difference between motile and immotile cells can be further studied in the future.

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