USING A THREE-CHAMBER CULTURE CHIP TO STUDY THE INTERACTIONS AMONG CANCER CELLS AND TWO TYPES OF STROMAL CELLS

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ABSTRACT

We employ a microfluidic cell culture chip with three culture chambers to investigate the interactions among cancer cells, macrophages and myofibroblasts. The channels connecting the chambers are controlled by pneumatic microvalves. We revealed that the conditioned media of macrophages and myofibroblasts have synergistic effects on accelerating the migration of cancer cells. However, as the myofibroblasts are pretreated with the conditioned medium of macrophages, the myofibroblasts' ability to enhance the migration of cancer cells is suppressed. We confirmed that the tumor necrosis factor-α produced by macrophages degrades the activities of myofibroblasts.

KEYWORDS: Myofibroblast, Tumor Associate Macrophage, Pneumatic Microvalve, Tumor Necrosis Factor

INTRODUCTION

The interactions between cancer cells and stromal cells in tumor microenvironment play crucial roles in cancer progression and metastasis. Among the stromal cells, cancer-associated fibroblasts and tumor-associate macrophages (TAMs) are of special importance [1,2]. The cancer-associated fibroblasts usually express the phenotype of myofibroblasts [3], which can enhance the migration ability of various cancer cells. On the other hand, TAMs are involved with tumor metastasis and angiogenesis. The coculture of cancer cells with either myofibroblasts or TAMs provides useful information for understanding the tumor microenvironment [4,5]. However, because the interactions between the cancer cells and stromal cells are usually in the form of complicated cytokine loops, we have to consider the effects from these two kinds of stromal cells on the cancer cells in both parallel and sequential manners to obtain clearer pictures of the mechanisms mediating cancer cell motility.

In this work we use a microfluidic culture chip that contains three chambers to coculture lung cancer cells, myofibroblasts, and macrophages in the same condition. The three chambers are connected by microfluidic channels such that the conditioned medium (CM) of one type of cells can be flowed to treat the other two. The flow sequences of the CM are controlled by pneumatic microvalves [6], such that the change in cancer cell migration speeds under the stimulations from both myofibroblasts and TAMs can be revealed.

EXPERIMENTAL

Figure 1 shows the culture chip. The whole chip is made of polydimethylsiloxane (PDMS) with the volume of each chamber 64 μl. The major advantages of this chip include: (1) one type of cells receive the cytokines produced by the other two types of cells at a controllable timing and duration, and (2) the concentrations of cytokines in the CM are preserved because of the high cell number-to-volume ratio (~1000 μl^{-1}). We also use COMSOL Multiphysics to simulate the mixing of cytokines when the CM from two chambers are flowed simultaneously into the third chamber [Fig. 1(b)]. The mixing is uniform such that we may assume that every cell in the third chamber experiences the same concentration of cytokines. For better cell adhesions, we coat the surface of culture chambers with fibronectin before seeding the cells.

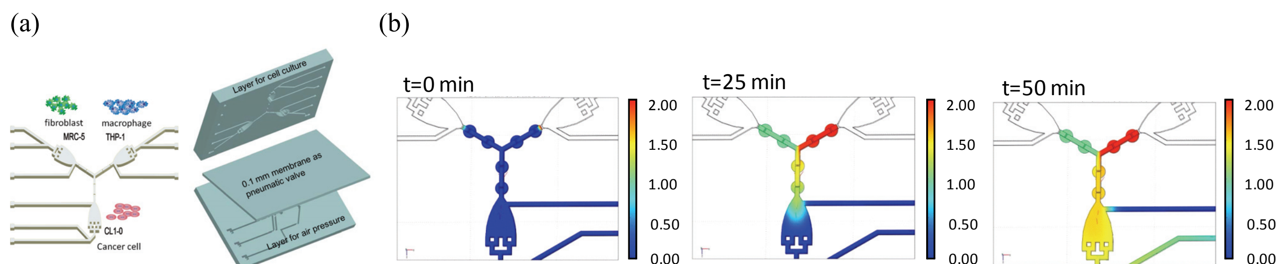

Figure 1: (a) Structure of the cell-culture chip used in this work. The whole chip is made of PDMS. (b) Simulation results of the cytokine mixing while the conditioned media from two chambers are flowed simultaneously into the third at a rate of 50 μl/h.

The cell lines used in this work are human lung cancer cells (CL1-0) [7], lung fibroblasts (MRC-5), and human macrophages activated from a monocyte cell line (THP-1) by phorbol myristate acetate. It has been verified that the macrophages obtained in this way express the functional profiles of M2 macrophages or TAMs, which can enhance tumor growth and invasion [8]. After seeding the cells into the culture chip, the fibroblasts are activated to be myofibroblasts by flowing the cancer cell CM to the chamber of fibroblasts for 24 hours [9]. All the cells are grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic pen-strep-ampho. After loading the cells into the chip, we replace DMEM with Leibovitz L-15 medium that is formulated for the use in CO_2-independent culture systems.

We determine the concentration of transforming growth factor-β1 (TGF-β1) in the myofibroblast CM by a sandwich enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (R&D Systems, Abingdon, UK). For this assay, the myofibroblasts are cultured in 35 mm dishes with a density ~500 cells/mm^2 overnight before we collect the CM.

RESULTS AND DISCUSSION

In a previous study we had confirmed that the CM of myofibroblasts, rather than fibroblasts, can increase the migration speed of cancer cells [9]. In this work, we compare the effects of the CM of myofibroblasts and that of the myofibroblasts pretreated with the CM of TAMs (called TAM-pretreated myofibroblasts) on the migration speed of cancer cells. Figures 2(a) and 2(b) show the results of the migration speed and proliferation of cancer cells. Compared with the myofibroblast CM, the medium of TAM-pretreated myofibroblasts has lower ability to accelerate cancer-cell migration, but higher capability to improve the proliferation. A very interesting point is that, the mixed CM of myofibroblasts and TAMs has the highest accelerating effect on cancer cells, as shown in Fig. 2(c). The results in Fig. 2(c) indicate that, although the CM of myofibroblasts and TAMs have a synergistic effect on accelerating the migration speeds of cancer cells, the CM of TAMs could simultaneously reduce this ability of myofibroblasts. In other words, as a tumor grows, the TAMs recruited into the tumor microenvironment can firstly reduce the cancer cell motility by suppressing the activities of myofibroblasts. This is a novel effect found in the functions of TAMs.

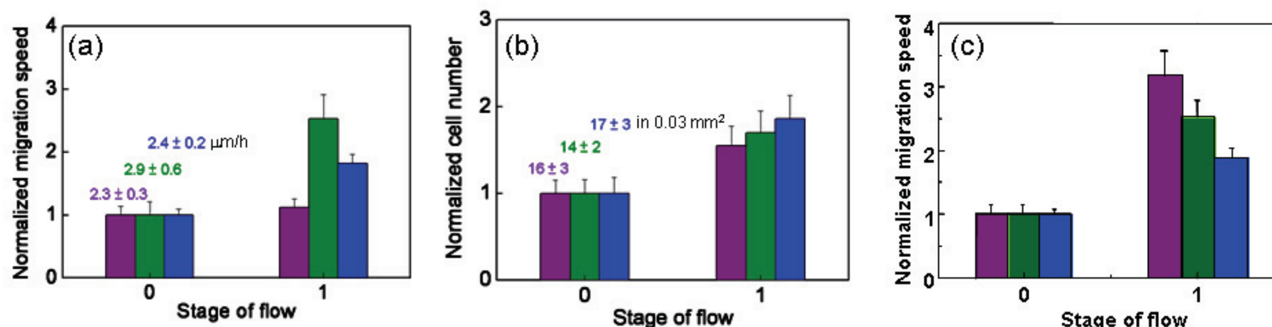

Figure 2: The effects of myofibroblast conditioned media on the migration and proliferation of cancer cells. Stage 0 represents individual culture, while in Stage 1 the flow is from myofibroblasts (or TAM) to cancer cells. In Panels (a) and (b): purple, empty medium; green, myofibroblast medium; blue, TAM-pretreated myofibroblasts medium. In Panel (c), purple, mixed myofibroblast and TAM medium; green, myofibroblast medium; blue, TAM-pretreated myofibroblasts medium. The values shown in the graph are the averages of 300 cells in each case.

In order to reveal how the TAM CM reduces the activities of myofibroblasts, we first check the change of α-smooth muscle actin (α-SMA), a major marker of myofibroblasts [10], in the TAM-pretreated myofibroblasts. Figure 3(a) shows that the fluorescence intensity of α-SMA with the treatment of the TAM CM is reduced by ~50% in comparison with that of myofibroblasts. It was recently reported that the α-SMA in myofibroblasts can be reduced by tumor necrosis factor-α (TNF-α), which is known to be produced by macrophages [11]. Therefore we postulate that the α-SMA in the myofibroblasts in the chip is also reduced by the TNF-α secreted by the TAMs. In a previous study using a two-chamber cell culture chip we had verified that the TGF-β1 produced by cancer cells can transform fibroblasts into myofibroblasts, and the myofibroblasts secrete TGF-β1 for self-sustaining [9]. Therefore we would also like to know if the TNF-α secreted by the TAMs has an antagonistic effect on TGF-β1 of myofibroblasts. We use ELISA to measure the concentrations of TGF-β1 in the CM of fibroblasts, myofibroblasts, and TAM-pretreated myofibroblasts. We also use a recombinant anti-human TNF-α antibody (MAB2101, R&D Systems, Minneapolis, MN) to neutralize the TNF-α in the CM of TAMs. The results in Fig. 3(b) confirm that the CM of TAMs reduces the level of TGF-β1 in the CM of myofibroblasts. Nevertheless, as the TNF-α in the CM of TAMs is neutralized, the TGF-β1 in the CM of myofibroblasts raises to the level close to that in the un-treated myofibro-

blasts. Therefore, the TNF-α in the CM of TAMs plays an essential role in reducing the activities of myofibroblasts in the tumor microenvironment.

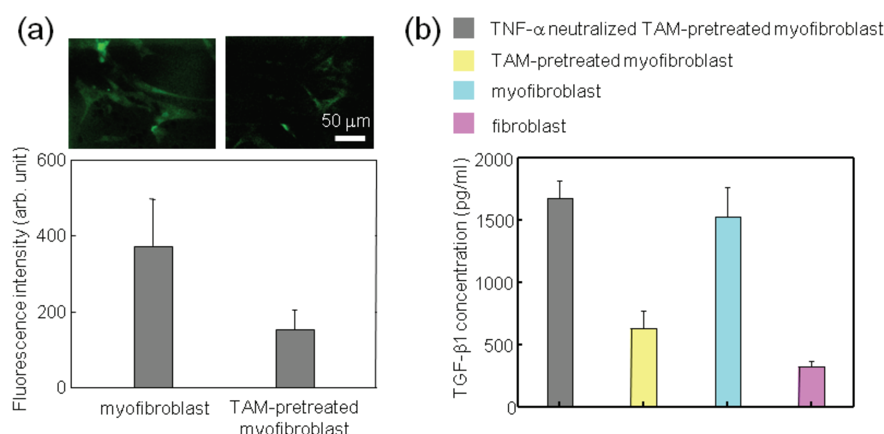

Figure 3: (a) The intensity of α-SMA in the myofibroblasts and TAM-pretreated myofibroblasts. The values are the averages of 100 cells. (b) The concentrations of TGF-β1 in the CM of fibroblasts, myofibroblasts and pretreated myofibroblasts measured by using ELISA. The values are the average of three repeated measurements. Error bar, standard error of the mean.

CONCLUSION

By using a three-chamber cell-culture chip we find that although the CM of myofibroblasts and TAMs have a synergistic effect on accelerating the migration of cancer cells, the CM of TAMs suppresses both the expression of α-SMA and the secretion of TGF-β1 in the myofibroblasts. The major effective constituent for this myofibroblasts antagonism from the TAMs is TNF-α. The three-chamber cell-culture chips can be used for assays of such complicated cell–cell interactions in precisely controlled timing sequences and help to reveal novel functions of the stromal cells in tumor microenvironment.

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