Monitoring single tumor interactions with fibroblasts in autophagy activation

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ABSTRACT

In tumor microenvironment, tumors interact with stromal cells such as immune cells, mesenchymal stem cells and fibroblasts etc. Specifically, communication with fibroblasts is related to tumor survival, proliferation and metastasis. We developed a high throughput screening platform based on a single tumor cell-to-fibroblasts interaction and monitored kinetics of GFP-LC3 positive fibroblasts by single tumor. Our research can have a potential to monitor single tumor which can activate autophagy in fibroblasts and help to study the heterogeneity of tumor and mechanism between tumor and fibroblast in autophagy.

KEYWORDS: Microfluidics, Single-cell level analysis, Tumor, Fibroblast, Autophagy

INTRODUCTION

Tumorigenesis consists of a complex environment with interaction between stroma, such as immune cells, mesenchymal stem cells and fibroblasts. Specifically, Interaction with fibroblasts plays a critical role in tumor proliferation and metastasis. With interaction between tumors and fibroblasts, fibroblasts transdifferentiate to carcinoma associated fibroblasts (CAFs) and autophagy in CAFs is induced. [1] Autophagy is a homeostasis mechanism with degradation of dysfunctional organelles in a stressful condition such as starvation, growth factor withdrawal and oxidative stress. Although autophagy of CAF helps to promote the survival and proliferation of cancer, the study about how tumor activates autophagy in fibroblasts is beginning step. We developed a high throughput platform to monitor the interaction between single tumor and multiple number of fibroblasts to screen the heterogeneity of single tumors and study about the mechanism of autophagy in communication between tumor and fibroblasts This device can be used to characterize the related gene of tumor in autophagy activation of fibroblasts and be useful system in the therapeutic field.

EXPERIMENTALS

We used a high throughput platform that consists of polydimethylsiloxane (PDMS) reservoirs and PDMS porous membrane and glass substrate. (Figure 1(B)) In case of PDMS porous membrane, its thickness is 50 μm and it has 1000 holes of 30 μm diameter, which is used for trapping single tumor cell. Bottom side of PDMS membrane was coated by fibronectin solution and reservoir was coated by PEO-PPO-PEO copolymer solution to prevent non-specific binding. GFP-LC3 transfected MEF cells are cultured on bottom side of PDMS membrane in incubator for overnight. With various conditions, we obtained 49 % single cell entrapment efficiency as optimized trapping efficiency. Using live cell imaging system, we can observe kinetics of autophagy activation in fibroblasts in hole with single tumor and empty hole and compare the ratio of autophagy activation in empty hole and hole with single tumor. We decided reasonable interaction duration as 6 hours when we can discriminate between empty hole and hole affected by single tumor. (Empty hole: 3.36 ± 1.39 % and Hole with single tumor: 6.41 ± 3.30, p-value < 0.05) Also, we did tracking GFP-LC3 positive fibroblasts ratio in each hole to know how different GFP-LC3 positive fibroblasts ratio in each hole is. It shows heterogeneity of single tumor cell in autophagy activation in fibroblasts. (3 independent chips, 67 holes with single tumor and 59 empty holes) (Data not shown in figures)

CONCLUSION

We developed a single cell level monitoring platform based on interaction with multiple cells. This platform visualized the interaction of one cell with a population of other cells, which mimic early stage of tumor microenvironment. Using live cell imaging system, we also monitored kinetics of GFP-LC3 positive fibroblasts by single tumor and tracked the change of ratio of GFP-LC3 positive fibroblasts ratio in each hole with various interaction duration. From this research, we can decide criterion to differentiate between the positive holes and negative holes and isolate specific tumor cells to do further study autophagy mechanism between tumor cell and fibroblast. Our research can study single-level tumor which can be related to autophagy activation in fibroblasts and study the mechanism between tumor and fibroblast in autophagy.

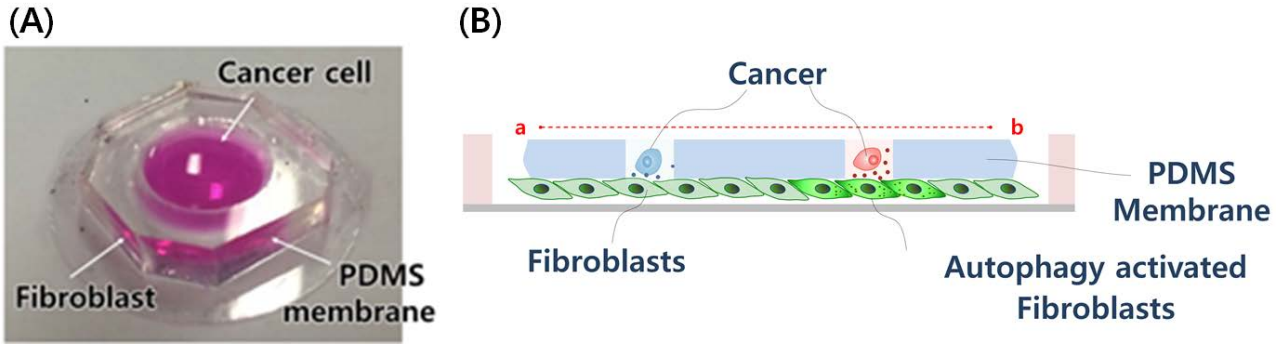

Figure 1. Microfluidic device for analysis of tumor-stroma communication through autophagy in fibroblast by secreted paracrine from cancer cells. A. *Image of the microfluidic screening device,* ***B.*** *Schematics of single tumor cell pairing with fibroblasts.*

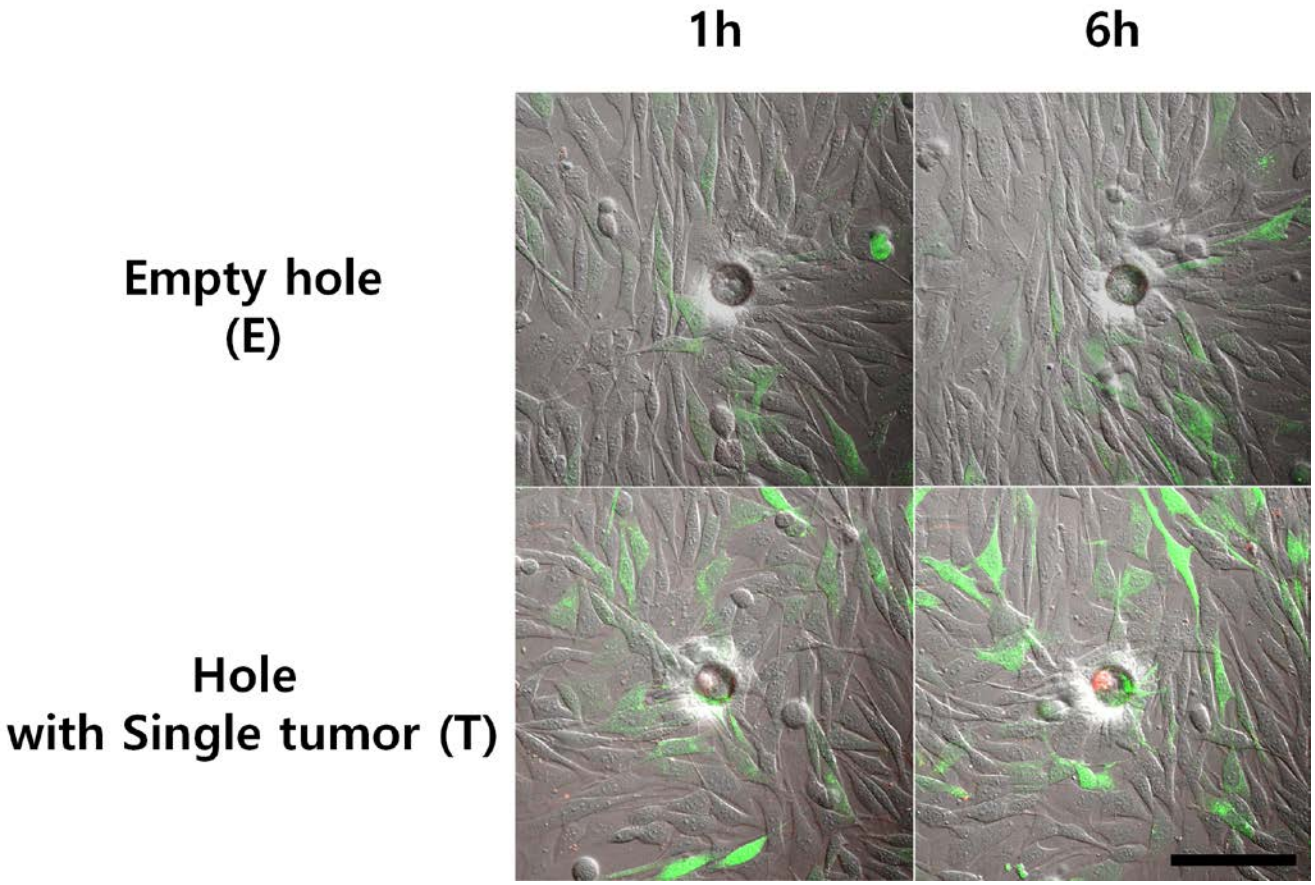

Figure 2. After 6 hour interaction duration, holes with single tumor are discriminable from empty holes in case of percentage of autophagy activated fibroblasts . *Images of dynamics of autophagy activation of fibroblasts near empty hole and hole with single tumor cell (GFP : LC3 protein in fibroblasts, RFP : MDA-MB 231, Scale bar :100μm*).

REFERENCE

[1] Bremnes et al., Journal of Thoracic Oncology 6, 209-217 (2011)