Transitions Between Epithelial and Mesenchymal States in Microfluidic Platform: Acquisition of Malignant and Stem Cell Traits

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ABSTRACT

Epithelial–mesenchymal transition (EMT) is an indispensable mechanism during morphogenesis, as without mesenchymal cells, tissues and organs will never be formed. Transitions between epithelial and mesenchymal states have crucial roles in embryonic development and without EMT, in which polarized epithelial cells are converted into motile cells, multicellular organisms would be incapable of progressing past the blastula stage of embryonic development. EMT provides a new basis for understanding the progression of carcinoma towards dedifferentiated and more malignant states. Some of these subpopulations may exhibit more differentiated features, whereas others have characteristics of stem cells. We have developed a 3D microfluidic system. The devices contain flow channels with each channel separated by a 3D collagen scaffold filled through other microchannels. We have studied migration into collagen scaffolds under a gradient in growth factor and also under various co-culture conditions.

Keywords: EMT, 3D scaffolds, Microfluidic devices, cancer treatment.

1 INTRODUCTION

In recognition of the importance of these tumor-associated phenotypes in metastasis and cancer-related mortality, targeting the products of such cellular plasticity is an attractive but challenging approach that is likely to lead to improved clinical management of cancer patients [1, 2, 3]. Emerging data suggest a role for these processes in regulating cellular plasticity in normal adult tissues and in tumors, where they can generate multiple, distinct cellular subpopulations contributing to intratumoural heterogeneity [4]. A need exists for a new high throughput for testing anti-metastatic drugs. Any drugs target either interavasation or exteravasation primarily for lack of an effective assay to use for this purpose. We have developed a 3D microfluidic system by integrating a hydrogel scaffold into a PDMS device for cell growth, with co-culture capability. This novel microfluidic platform has proven to be a versatile and powerful tool to study cell migration for various biological applications. It provides a well-controlled cell culture environment, which can be observed in real time. Furthermore, it allows for integration of biophysical and biochemical factors, essential in mimicking physiological conditions as cells constantly receive signals from both their soluble and insoluble environments. We are now exploring new applications with this platform as a model system for transitions between epithelial and mesenchymal states [4].

1.1 Proposed approach

The technology has shown promise in additional venues requiring a model ECM, chemotactic gradient, and cell-cell interactions but we have conducted limited studies on cancer cell migration and specially EMT processes. This technology requires further development before it can be applied to the quantitative assessment of molecular and cellular level behavior metastatic potential in a clinical setting. Here we describe the initial work that led to the present research plan and demonstrate the potential of our Microfluidic technology.

2 THE 3D MICROFLUIDIC PLATFORM

The Microfluidic device contains two or three independent flow channels separated by a 3D gel scaffold as illustrated in figure1. Microfluidic devices are widely used in bioengineering research offering a powerful platform for the performance of assays. In microfluidic systems, small volume of solvent, sample, and reagents are moved through microchannels embedded in a chip and miniaturized biological assays into these chips include DNA sequencing, polymerase chain reaction, electrophoresis, DNA separation, enzymatic assays, immunoassays, cell counting, cell sorting, and cell culture.

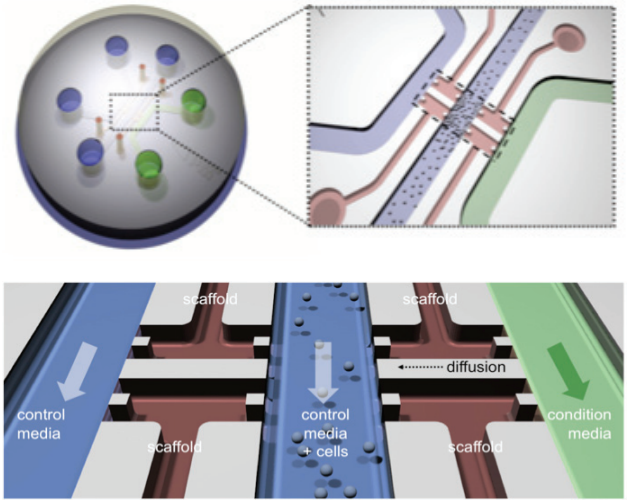

Figure 1: Three channel device capabilities include simultaneous 2D and 3D culture, excellent imaging, multi-culture up to 5 different cell types, concentration gradients and flow, hypoxia**.**

Poly (dimethylsiloxane) (PDMS) elastomer is a specially well–suited material for the fabrication of microfluidic devices for biological assays purposes since it is inexpensive, flexible, optically transparent (and compatible with most of the optical methods available for detection), impermeable, non–toxic to cells, permeable to gases and easily bonded to other surfaces.

3 RESULT AND DISCUSSION

Although EMT processes are documented in many in vitro cancer cell model, the significance of EMT during cancer progression and even its relevance in human cancer tissues has remained a matter of debate until very recently. This resistance was mainly due to the lack of convincing evidence of EMT in clinical samples. EMT is central to both physiological and pathological processes, and pathological EMT can be regarded as a reactivation of developmental program in the adult.

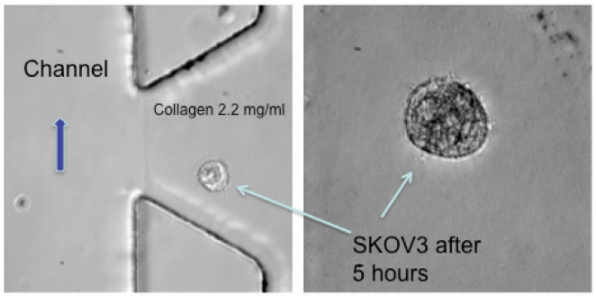

Figure 2: The aggregate cancer cells were suspended in collagen in 3D region of Microfluidic device

3.1 HGF induced aggregate A549 (human lung cancer cells) in 3D matrix

It has been proved that HGF is able to increase the invasive potential of primary lung and ovarian cancer cells on 2D surfaces. To establish a 3D invasion model, we embedded aggregate cancer cells in collagen gel (2.5 mg/mL), and loaded the mixture into scaffold chamber into our devices figure 2. Collagen in scaffold region area served to be ECM, not only supporting 3D distribution of aggregate cells but also allowing nutrient exchange between gel and perfusion channels figure 3.

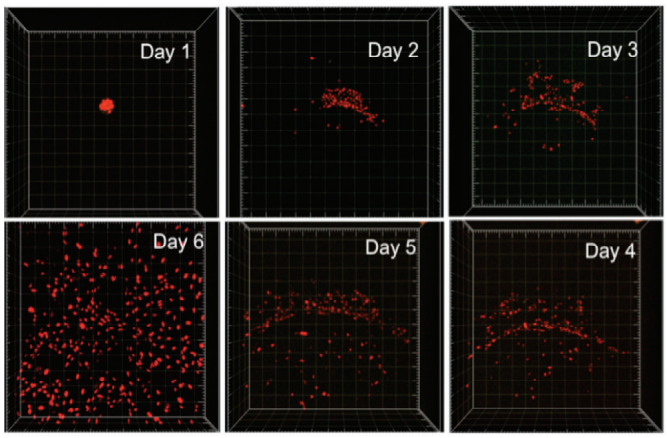

Figure 3: Aggregate A549 lung cancer cells are suspended in collagen and expose HGF during to study EMT.

3.2 Cancer cell (aggregate A549, human lung cancer cell) interaction with an Endothelial (HUVEC) cell layer

Interactions between tumor (aggregate) cancer cells and an intact EC layer have been observed in our systems. While recognizing that A549 (lung cancer cells) are known to EMT process, they stimulate to migrate and interact with an EC layer in the model system as shown in figure 4. The H2B expressing A549 cells migrated through collagen gel toward the endothelial monolayer (GFP expressing HUVEC) making contact through a cellular extension. The system shows clear potential to explore migration and intravasation of cancer cells.

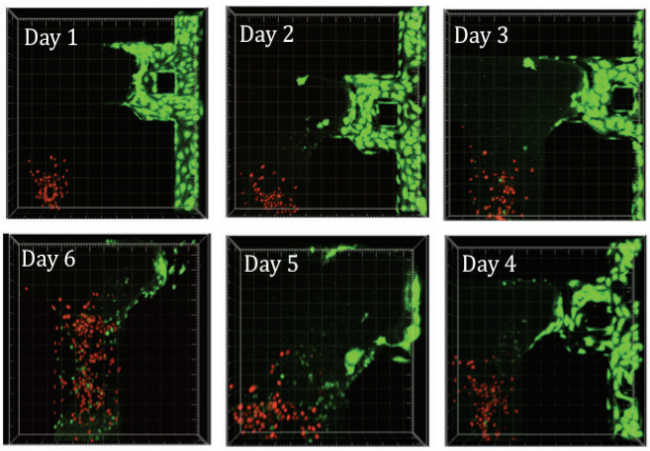

Figure 4: Interaction between A549 and EC during EMT

4 FUTURE WORKS

The *in vitro* models that are used to study EMT suffer from several limitations. Few common carcinoma cell types with a well-defined epithelial phenotype can complete EMT in vitro perhaps because EMT is very sensitive to culture conditions, including substrates and the presence of serum. This method dose not only facilitates tumor spheroid formation, but also enables to establish communication between co-culture cells via medium diffusing in matrix. This co-culture system would be invaluable in modeling cancer progression and testing therapeutics in biologically relevant context. The established co-culture model should be applicable for analysis of the invasion mechanism and testing other anti-invasion agents as well.

REFERENCES

[1] J. P. Thiery and J. P. Sleeman. Complex network orchestrate epithelial-mesenchymal transition. Nature Reviews/ Molecular and cell Biology 7, 131-142, 2006.

[2] J. P. Thiery. Epithelial-Mesenchymal Transitions in Tumor Progression. Nature 2, 442-454, 2002.

[3] J. M. Lee, S. Dedhar, R. Kalluri, and E. W. Thompson. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. The Journal of Cell Biology 172, 973-981, 2006. .

[4] S. Chung, R. Sudo, P. J. Mack, C. R. Wan, V. Vickerman and R. D. Kamm. Cell migration into scaffolds under co-culture condition in a Microfluidic platform. Lab on a Chip 9, 269-275, 2008.