# MESENCHYMAL STEM CELLS PROMOTE THE INVASION IN SALIVARY GLAND CANCER ON THE BIOMIMETIC MICROSYSTEM

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**ABSTRACT**

As the participant of the tumor microenvironment, mesenchymal stem cells (MSCs) play an important role for further understanding the tumor progression, which gradually become the hotspot in tumor biology [1, 2]. In this work, we investigated the heterotypic interaction between MSCs and epithelial salivary gland cancer (ACC-M) in an in-vivo like tumor microenvironment by using a biomimetic microfluidic device. We also explored the involvement of CXCL12/CXCR4 signal pathway in the interaction between MSCs and ACC-M cells, in order to probe the role of MSCs in epithelial tumor.

**KEYWORDS**

Biomimetic, mesenchymal stem cells, tumor cells

## INTRODUCTION

Mesenchymal stem cells (MSCs), which are the progenitors of stromal cells and fibroblast, have been found to interact with multiple types of tumor. The relationship between MSCs and cancer is becoming the subject of an increased interest in cancer biology [3], and it is expected to bring new topics to an old disease and hopefully reveal new ideas for effective treatment. The current emerging research on the participation of MSCs in cancer progression appears to involve two contradictory aspects [2]. On the one hand, MSCs can specifically home to the sites of injury as well as tumors, holding a promise avenue for tumor-targeted delivery of therapeutic agents. On the other hand, MSCs can be abused by cancer cells and have tumor-promoting properties. It is worthy of note, more investigations are required to deeply elucidate the dual role of MSCs participating in cancer progression and treatment in a more in-vivo like cancer environment.

Overall, the evidence for MSCs as active participants in cancer is just emerging, with more questions raised than answered. Several critical issues are deserved to be concerned. For example, whether the current models used in the studies can accurately reflect what is actually happening in the natural setting. In addition, since the present reports have been limited to only a few types of cancers, it is still unknown how MSCs interact with other types of cancers. Adenoid cystic carcinoma (ACC) is an aggressive malignant epithelial tumor arising from salivary glands, and it is mainly characterized by the ability to disseminate to the distant sites in the body, particularly, the lung and the liver [4]. Treatment of ACC is often frustrating because of its multiple recurrences and distant metastasis. As the progenitor cells of the stroma, MSCs are adult stem cells, which have received much interest of late since the demonstration of clonal multipotency [5], however, the relationship between MSCs and ACC is poorly understood.

Recent development in microfluidic technology has allows for the creation of physically relevant tumor microenvironment by controlling the biochemical factors that influence cellular behaviors temporally and spatially, and manipulating the multiple cell types in a natural setting 20-22. In this work, we aim to explore the role of mesenchymal stem cells in salivary gland cancer by using the biomimetic microfluidic device.

## EXPERIMENT

The three dimensional co-culture device used for investigating the heterotypic interaction between MSCs and ACC-M cells is composed of six co-culture units integrated with two side medium channels, as shown in figure 1. Each co-culture unit includes two round chambers (A and B) adjacent with each other. The height of chamber A was designed to be lower than that of chamber B and medium channels (Figure. 1a), so that the two different types of cells can be introduced into the chambers sequentially. The two medium channels flanked aside could provide enough nutrients for cell co-culture.

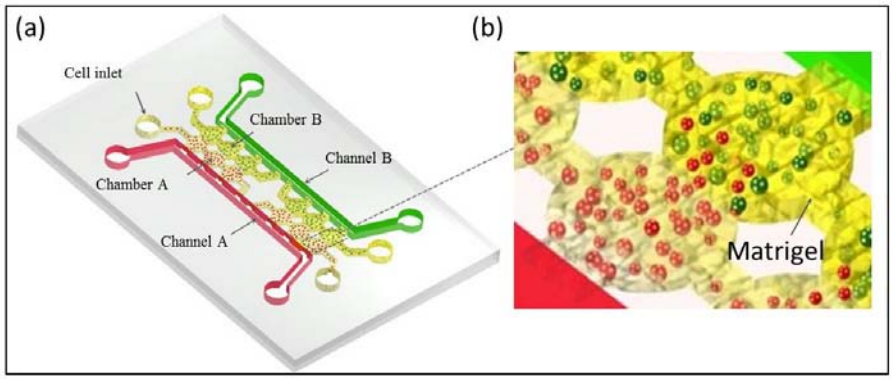


*Figure 1: Schematic of the biomimetic microdevice and the magnification illustration of a coculture unit encapusalting different types of cells.*



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For 3D indirect contact co-culture, MSCs could spread thinner protrusion and migrated into the 3D matrix encapsulated with ACC-M cells in a time-dependent manner (Figure 2a). This homing phenomenon was also observed in 3D direct contact co-culturing assay as shown in figure 2b. MSCs extended protrusions, moved into the periphery of ACC-M spheroids and squeezed into the ACC-M spheroids. With the increase of culturing time, the ACC-M spheroids were gradually dispersed by MSCs and exhibited loose cell-cell contact. The E-cadherin expression was significantly reduced after cultured with MSCs for 48h (Figure 2c). These results suggested the specific effect of MSCs on ACC-M cells by disrupting the cell-cell connection in tumor spheroid. In vivo, the biological process of MSCs homing to tumor microenvironment is the fundamental step to participate in tumor progression, which may activate the subsequent collective cell invasion and dissemination. It is assumed that MSCs might generate some soluble factors diffusing into extracellular matrix which might trigger certain signaling pathway in ACC-M cells and contribute to the establishment of distant metastasis by disrupting the cell-cell connection in tumor spheroids.

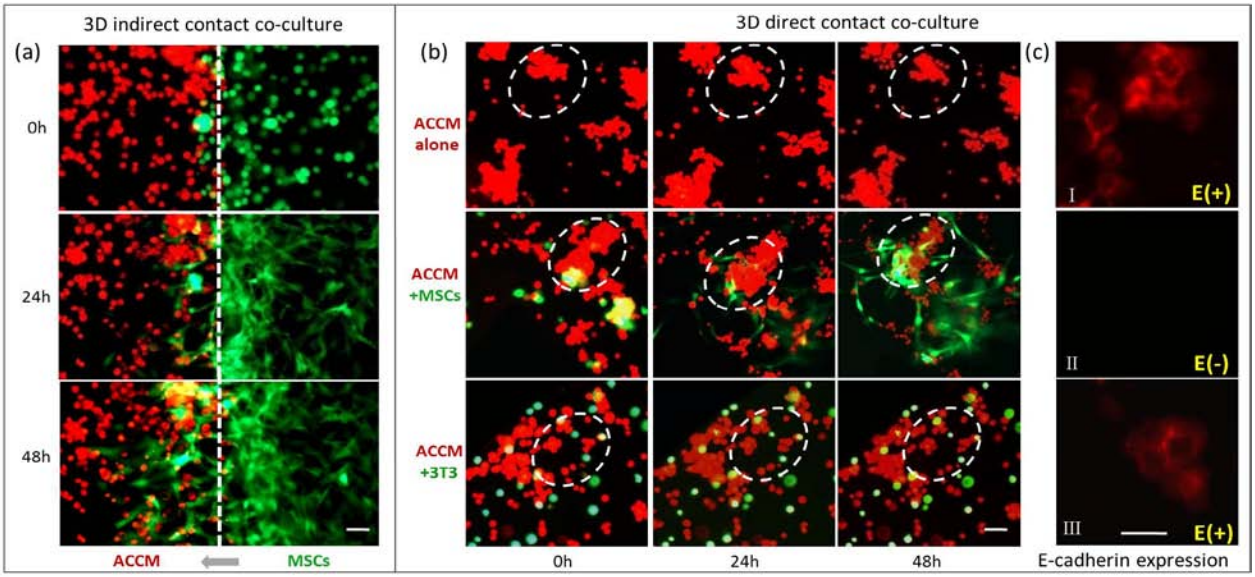


*Figure 2: Interaction between MSCs and ACC-M cells in 3D assay. (a). Fluorescent images of MSCs homing to ACC-M cells at different time). (b). Comparison of cell-cell connection in ACC-M spheroids when they are in physical contact co-cultured with MSCs and NIH3T3. (c). E-cadherin Expression I. ACC-M alone; Ⅱ. ACC-M and MSCs, Ⅲ. ACC-M and NIH3T3. (scale bar=50μm)*

The gradient of CXCL12 can be generated across the adjacent chamber after continuous perfusion of solution along the two side channels (Figure 3a-b). ACC-M cells were found to exhibit a high expression of CXCR4 on the cellular surface (Figure 3c). Under CXCL12 gradient, ACC-M cells were observed to leave the original site, migrating towards the high concentration of CXCL12, and invaded into the adjacent extracellular matrix obviously. This behavior could be inhibited by blockage of CXCR4 signaling using a CXCL12 antagonist (AMD 3100). The invasion ability of ACC-M cells under CXCL12 gradient was enhanced significantly after the addition of MSCs by characterizing the invasion distance of ACC-M in extracellular matrix (Figure 3d). We speculated that MSCs produced some soluble factors to trigger the CXCL12-CXCR4 signaling pathway in ACC-M cells, enhancing the metastasis process of ACC-M cells. The further work will conduct the exploration of ACC invasion in a multiple-factor interplay system, and elucidate the complex signal pathways in the MSCs-regulated ACC invasion. Together with previous work, we have emphasized the promoting role of MSCs in tumor metastasis and addressed the importance of MSCs-cancer biology in target therapy. In vivo, there has been much evidence that cell-cell interactions and mechanisms are highly specific not only for a particular organ system, but even for a particular histological cancer type. The current new findings of the contribution of MSCs in ACC invasion could shed light on the thinking of cancer evolution and MSCs-based cancer therapy. We thought that if tumor cells could be subsequently deprived of such signals secreted by stromal cells, especially the effect of MSCs, they might be able to revert to an earlier phenotypic state, in which they no longer display the traits of high-grade malignancy. Meanwhile, it reminded us to re-examine the safety of using MSCs as delivering vehicle for tumor-targeted therapy seriously.

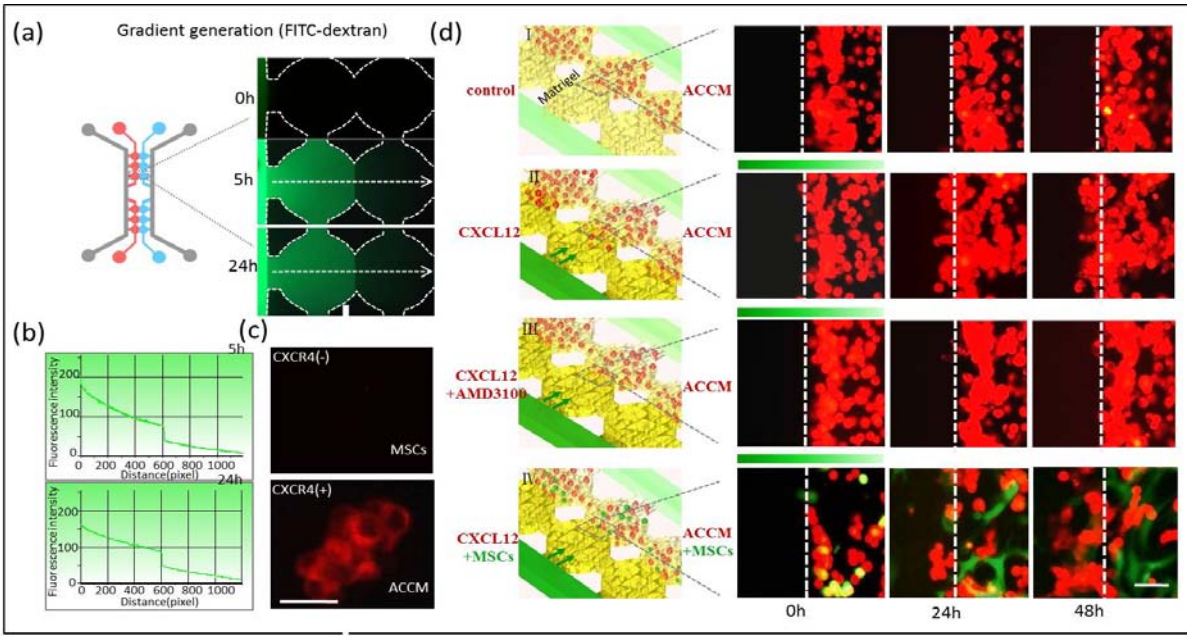


*Figure 3: The effect of MSCs on the invasion of ACC-M cells under CXCL12 gradient in 3D assay. (a-b): Characterization of the stability of CXCL12 gradient; c: CXCR4 expression in MSCs and ACC-M cells respectively; d: Comparision of the invasion in ACC-M cells under CXCL12 gradient in co-culturing with and without MSCs.(scale bar=50μm)*

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