Micropillar-arrayed surfaces promote transforming growth factor beta 1 induced epithelial to mesenchymal transition by focal adhesion kinase-related signaling in A549 cells

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To the Editor: Substrate rigidity and topography are two important biomechanical factors that can modulate the biological behavior of cancer cells such as epithelial to mesenchymal transition (EMT). Currently, although studies have begun to focus on how the mechanical microenvironment determines the fate of multiple cells, the mechanism of the mechanical microenvironment affecting EMT is still unclear.

Recent studies show that matrix mechanical signals played an important role in regulating EMT processes associated with disease development.[1-3] Several experiments using polyacrylamide hydrogels to change the rigidity of the substrate demonstrate that the rigidity of the substrate affects the EMT process. The key issue in the response of cells to physical environment stimulation is how cells convert mechanical signals into biological signals. The cell sensing of substrate's mechanical signals is mainly achieved by the cytoskeleton. The focal adhesion complex is the connection between the cytoskeleton and the substrate. Focal adhesion kinase (FAK) is located at the junction of multiple mechanical signal transduction pathways in the cells and activates multiple mechanical signal transduction pathways.[4,5] FAK related pathways are also associated with the EMT process.

In our previous work,[6] we confirmed the topography-induced changes in cell morphology, but we do not know which signals caused such changes in the cell. Although we have previously known that matrix topography and rigidity are synergistic with each other in inducing the EMT process, and the activation of the phosphatidylinositol 3-kinase/protein kinase B signaling pathway may be the cause of the mechanical environment-induced EMT. However, we do not know whether changes in the individual topology will affect the EMT process.

The study was carried out in the Key Laboratory of Biorheological Science and Technology, College of Bioengineering, Chongqing University from 2016 to 2019. We used micropillar-arrayed substrates formed by polydimethylsiloxane (PDMS) to investigate the effect of topography on biological behavior of human adenocarcinoma A549 cells. To test whether extracellular matrix topology affects the morphology of A549 cells, we used the fabrication process to make micropillar-arrayed substrates [Figure 1A and Supplementary Figure 1A, http://links.lww.com/CM9/A345]. As shown in Figure 1B and Supplementary Figure 1B, http://links.lww.com/CM9/A345, A549 cells in the PDMS planar and PDMS micropillar-arrayed substrates have different morphology. On the planar substrate and the pillar (10.2) ("10" stands for the diameter is 10 μm and "2" stands for the height is 2 μm) substrate, A549 cells were round and the cells were directed in multiple directions. However, on the substrate of pillar (10.4) and pillar (10.7), A549 cells were fusiform and spread to both sides. Also, micropillar-arrayed substrates affects the A549 cytoskeletal and focal adhesions organization. Vinculin expression was measured by immunofluorescence after A549 cells were cultured on different substrates for 48 h [Figure 1C]. As the spacing between the micropillar increases, the amount of fluorescence of the vinculin protein gradually increases [Supplementary Figure 1C, http://links.lww.com/CM9/A345]. As the spacing between the micropillars becomes larger, the depolymerization phenomenon of the cytoskeleton becomes more and more obvious [Figure 1D], which shows that the cells are more likely to migrate as the spacing of the micropillars increase. As shown in Figure 1E and

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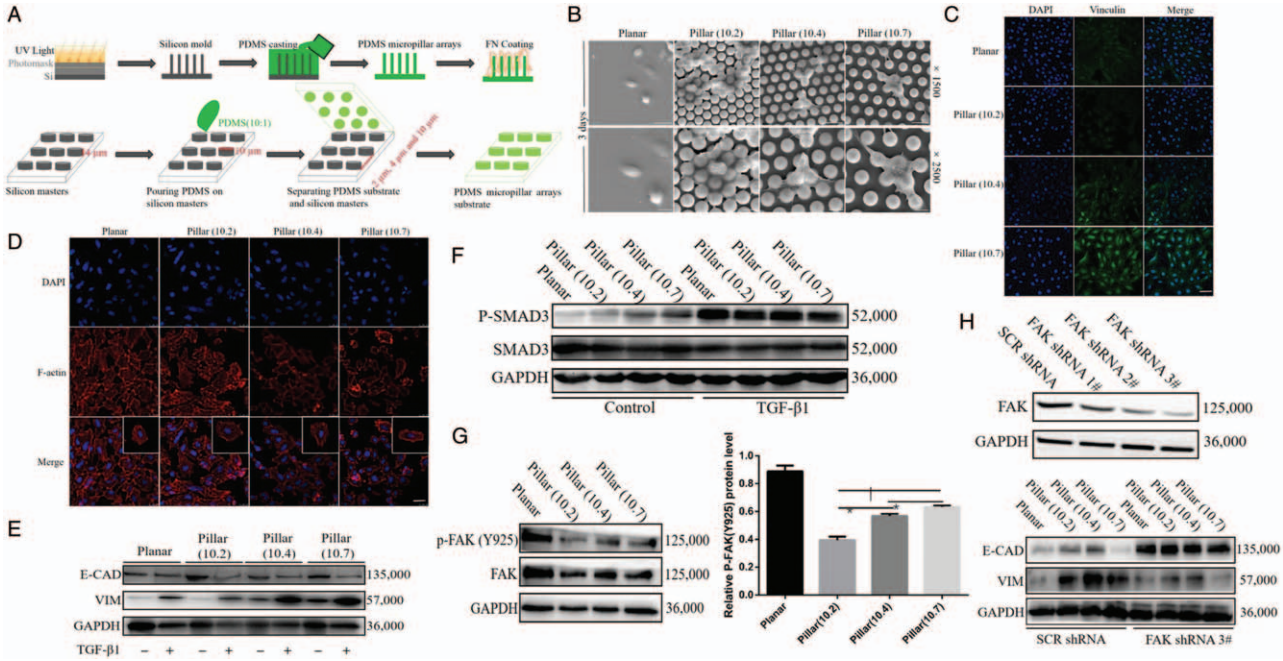

Figure 1: Micropillar-arrayed surfaces affected TGF-β1 induced epithelial to mesenchymal transition in A549 cells. (A) Schematic diagram of the PDMS micropillar-arrayed substrate. (B) SEM images of A549 cells cultured on micropillar-arrayed substrate. Scale bars in ×1500 original magnification are 20 μm and those in ×2500 original magnification are 10 μm. (C) Representative immunofluorescence images of vinculin (green) and DAPI (blue) in A549 cells cultured for 2 days on micropillar-arrayed substrate. Scale bar = 50 μm. (D) The cytoskeleton was specifically stained with a rhodamine-labeled F-actin. Scale bar = 20 μm. (E) The effect of micropillar-arrayed substrate on the process of EMT in A549 cells analyzed by Western blot. (F) The effect of micropillar-arrayed substrate on the expression of SMAD3 protein in A549 cells detected by Western blot. (G) Effect of micropillar-arrayed substrate on the expression of FAK and p-FAK protein in A549 cells. (H) FAK expression in A549 cell lines treated with scramble shRNA or FAK shRNAs and EMT-associated marker proteins in different A549 cell groups were analyzed by Western blot. Values are mean ± standard deviation, $n = 3$. $^{*}P < 0.01$; $^{\dagger}P < 0.001$. DAPI: 4', 6-Diamidino-2-phenylindole; EMT: Epithelial-mesenchymal transition; FAK: Focal adhesion kinase; PDMS: Polydimethylsiloxane; SCR: Scramble; SEM: Scanning electron microscope; shRNA: Small hairpin RNA; TGF-β1: Transforming growth factor beta 1.

Supplementary Figure 1D, http://links.lww.com/CM9/A345, when transforming growth factor beta 1 (TGF-β1) was not added, the expression of E-cadherin (E-CAD) decreased with an increase in spacing between micropillars and the expression of vimentin (VIM) increased significantly [Supplementary Figure 1E, http://links.lww.com/CM9/A345]. This process was enhanced by phosphorylation of SMAD3 and FAK [Figure 1F, Figure 1G, and Supplementary Figure 1F, http://links.lww.com/CM9/A345]. In addition, we infected A549 cells with a lentivirus expressing *FAK* small hairpin RNA (shRNA) [Supplementary Table 1, http://links.lww.com/CM9/A345 shows the sequences of shRNAs] and screened a cell line stably silencing the *FAK* gene. As shown in Figure 1H, after silencing the *FAK* gene, the expression level of E-CAD was relatively increased, and the expression levels of VIM was relatively decreased [Supplementary Figure 1G, http://links.lww.com/CM9/A345].

Taken together, our study reported the effect of topography on the development of A549 cells. A549 cells exhibited significantly different biochemical behaviors in different micropillar-arrayed substrates. In particular, as the spacing between the micropillars changed, the A549 cells began to express EMT-like behaviors. Similarly, the expression of cell adhesion-associated proteins and cytoskeletal proteins also changed as the micropillar spacing changed. In addition, micropillar spacing also affected the TGF-β1-induced EMT process, which is associated with the phosphorylation levels of SMAD3 and FAK. These findings indicated that the micropillar-arrayed substrate could affect the biological behavior of A549 cells and participate in the regulation of the EMT process. Collectively, these findings provide a new perspective for finding pulmonary fibrosis therapeutic targets.

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Conflicts of interest

None.

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Corrigendum

Corrigendum: Breast cancer immunology and immunotherapy: targeting the programmed cell death protein-1/programmed cell death protein ligand-1

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In the article "Breast cancer immunology and immunotherapy: targeting the programmed cell death protein-1/programmed cell death protein ligand-1" which appeared in vol. 133, issue 7, page 853 of *Chinese Medical Journal*,[1] the authors "Jing Zhao[1,2], Jian Huang[2,3]" should be corrected as "Jing Zhao[1], Jian Huang[2]", and their affliations "[2]Key Laboratory of Tumor Microenvironment and Immune Therapy of Zhejiang Province, Hangzhou, Zhejiang 310009, China; [3]Department of Breast Surgery, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009, China" should be corrected as "[2]Key Laboratory of Tumor Microenvironment and Immune Therapy of Zhejiang Province, Department of Breast Surgery, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009, China".

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1. Zhao J, Huang J. Breast cancer immunology and immunotherapy: targeting the programmed cell death protein-1/programmed cell death protein ligand-1. Chin Med J 2020;133:853–862. doi: 10.1097/CM9.0000000000000710.