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세뇨관 상피 세포의 상피-중간엽 전이에 대한 원자현미경을 이용한 정량적 연구

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Observation of Angiotensin II-induced epithelial-to-mesenchymal transformation in Tubular Epithelial Cells Utilizing Atomic force microscopy

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Epithelial-to-mesenchymal cell transformation (EMT) is the trans-differentiation of tubular epithelial cells into myofibroblasts, and is now recognized as a key contributor of renal fibrosis. It is generally known that angiotensin II (Ang II) plays a direct profibrotic role in the kidney. Many signalling pathways of Ang II have been discovered, but mechanical aspects have not yet been investigated. Atomic force microscopy (AFM) has become an important device to visualize various cells and biological materials for non-invasive imaging. In this study, we observed structural and mechanical changes in tubular epithelial cell after Ang II treatment using AFM.

The epithelial cell line NRK52E were stimulated with Ang II (10-6 mol/l) and Ang II±telmisartan (10-6 mol/l, a selective AT_1 R antagonist) or Ang II±blebbistatin (myosin II inhibitor, 10-6 mol/l). Atomic force microscopy was performed to measure cellular stiffness, cell volume and roughness of cell surface. EMT markers were determined.

After 24 hr of Ang II stimulation, cells have transformed to a flattened and elongated mesenchymal morphology as is characteristic for EMT. AFM was able to detect nodular protrusions of around the cell junctions, where stress fibers of neighbouring cells make contact. F-actin staining of Ang II stimulated cells shows an increase of stress fiber formation all over the cell area and an intense bundling at the cell junctions. To quantify cell stiffness, we calculated the cellular spring constant, Kcell, by modeling the cell-tip interaction as two springs. NRK52E stimulated with Ang II showed a significant increase in spring constant ($p<0.001$, n=30). After 24 hr of Ang II exposure, the roughness of cell surface at junctional area and cell volume were significant increase ($p<0.001$, n=30). However, coincubation of Ang II with either telmisartan or blebbistatin attenuated the Ang II-induced increase in cell stiffness as well as the Ang II-induced increase in cell volume and roughness of cell junction. Ang II induced a increase in α-SMA and a reduction of E-cadherin, as determined by immunocytochemistry. In contrast, Ang II-induced changes in α-SMA and E-cadherin were completely blocked by treatment with telmisartan or blebbistatin.

The present study shows an increase cell stiffness and roughness during EMT as a consequence of stress fibre formation. We observed structural changes in tubular epithelial cell induced by Ang II, and cytoskeletal dynamics of EMT using AFM

Key Words: 상피-중간엽 전이, 원자힘 현미경, 안지오텐신 II
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