CYTOSKELETON DYNAMICS AND SPATIAL ORGANIZATION DURING EPITHELIAL-TO-MESENCHYMAL TRANSITION

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Epithelial-to-mesenchymal transition (EMT) is a crucial process that occurs during normal development, such as embryogenesis and organ formation. If it is inappropriately activated, it can lead to pathological processes like metastasis. Although EMT is well studied at the morphological and transcriptome level, cytoskeleton changes during this process are less understood. In our work we aimed to describe the morphological changes that occurred in MCF-7, A-549 and HaCaT cells after EMT, analyze microtubule dynamics, spatial distribution and its contribution to cell motility, identify changes in actin filament organization and study focal adhesion turnover in the cells after EMT.

We hypothesized that the dynamics of microtubules in cells undergoing EMT might change. Cells undergoing EMT were expected to have more dynamic microtubules. Also, cells undergoing EMT are expected to adhere more efficiently to diverse substrates, and therefore spread and move more easily. Focal contacts in cells undergoing EMT were expected to be more pronounced and dynamic than in cells that not undergoing EMT.

The research methods used in the study included inducing EMT in modified MCF-7 cells through an inserted inducible Tet-on system and stimulation of EMT in A-549 and HaCaT cells using TGF-β. The cells were observed using bright field microscopy, and immunofluorescence analysis was conducted to visualize microtubules and actin filaments. Transfection with EB-3–RFP protein was done to describe and measure microtubule dynamics, while transduction with Talin-RFP and transient transfection with Ptag-RFP-vinculin was done to visualize focal adhesions. Time-lapse fluorescent microscopy was used to record films, and the Fiji Image J program was used to analyze the data. All statistical analysis was performed using GraphPad Prism (Dotmatics, USA), and a nonparametric Mann-Whitney U test or parametric t-test with Welch correction. The actin filament measurements were completed using Matlab scripts.

The major research findings: In all three-cell models after EMT, cell morphology changed. Cells increased in size. MCF-7 and HaCaT became spread out, while A-549 became elongated. Some of the cells lost cell-cell contacts. All three cell models after EMT had alterations in microtubule organization and dynamics. MCF-7 and HaCaT cells showed more frequently individual MTs at cell edges, while A-549 had less covered nucleus by MTs after EMT. Microtubule dynamics (growth velocity) increased, and the length of microtubule growth tracks became longer. The average angle of MT growth trajectories to cell radius decreased. Actin fibers rearranged into more pronounced stress fibers after EMT. Alterations in the focal adhesion behaviors were different in three models used. These results indicate that cytoskeletal changes during EMT mainly include increased microtubule dynamics.