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ADAPTOR PROTEIN RuK/CIN85 INDUCES EPITHELIAL-TO-MESENCHYMAL TRANSITION IN HUMAN A549 LUNG ADENOCARCINOMA CELLS

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Introduction. Due to its aggressiveness, non-small cell lung cancer (NSCLC) remains the major cause of cancer-related mortality through the world. The reversible process of epithelial-to-mesenchymal transition (EMT) is responsible for increased migration and invasiveness of cancer cells and is also important in metastasis. Interplay between extracellular signals and consequent modulation of receptor-mediated signaling networks provides a fine control of morphofunctional changes associated with EMT. Cells undergoing EMT lose expression of epithelial markers (e.g., E-cadherin) and gain expression of mesenchymal markers (e.g., vimentin) through differential expression and activation of transcription factors including Twist1, ZEB1, ZEB2 and Snail. The pro-oncogenic adaptor protein is a multi-modular scaffold protein that regulates spatiotemporal organization of supramolecular signaling complexes involved in the control of cell proliferation, migration, invasion and metastasis. In the present work, human A549 lung adenocarcinoma cell line was used as a model of NSCLC to study the role of Ruk/CIN85 in EMT *in vitro*.

Methods. Sublines of A549 cells overexpressing Ruk/CIN85 were obtained by transfection with pRc/CMVRuk1 plasmid. Ruk/CIN85 expression in A549 cells was suppressed by infection with lentivirus encoding Ruk/CIN85-specific shRNA. Stable transfectants/infectants were obtained by selection in the presence of specific drugs, G418 and puromycin, respectively. Expression levels of Ruk/CIN85 in modified cells were determined by both Western-blot analysis and qRT-PCR. Proliferation was evaluated by direct cell counting with trypan blue and MTT test. Profiling of EMT-related transcription factors mRNAs expression was studied using qRT-PCR.

Results. At first, A549 cells sublines with different Ruk/CIN85 expression levels were generated. It was demonstrated that cells with Ruk/CIN85 overexpression proliferate faster than control cells; on the contrary Ruk/CIN85 suppression led to a decrease in cell proliferation. There was a clear correlation between the level of Ruk/CIN85 expression and morphological changes in obtained sublines. Cells with Ruk/CIN85 up-regulation acquired the features inherent of the mesenchymal phenotype whereas adaptor protein suppression resulted in epithelial phenotype. The results of qRT-PCR analysis showed that Ruk/CIN85 overexpression in A549 cells led to the 10-fold increase in the expression levels of Twist1 and Zeb2 mRNAs. At the same time, Ruk/CIN85 knockdown resulted in a decrease of Zeb2 and vimentin mRNAs and simultaneous increase of Snail1 mRNA level.

Conclusions. The obtained results pointed to the potential regulatory role of Ruk/CIN85 in mechanisms underlying EMT in NSCLC.

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