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EPITHELIAL-MESENCHYMAL TRANSITION IN MELANOMA PROGRESSION: THE CONTRIBUTION OF ADAPTOR PROTEIN RUK/CIN85

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Adaptor proteins of multi-modular structure are essential components of signaling networks. By interaction with other proteins, nucleic acids and lipids, they serve as frameworks for multimolecular complexes assembly [1]. It allows them to play a crucial role in signal transduction. Therefore, genetic alterations in the structure of such important cellular regulators and changes in their expression can lead to signaling disturbances, which may result in the emergence of numerous diseases, including cancer. Reversible process of epithelial-mesenchymal transition (EMT) has been recognized as driving force in metastasis of epithelial cell malignancies. The progression of melanoma, like that of carcinoma, is also characterized by a "phenotype switching" pattern reminiscent of EMT [2].

Aim. In light of the reported relationship between primary melanoma stages and the adaptor protein Ruk/CIN85 expression levels [3], the purpose of this study was to test the hypothesis that Ruk/CIN85 overexpression/knockdown in melanoma cells may be involved in the regulation of EMT.

Materials and methods. The mouse melanoma cell line B16-F10 and its sublines with up-/down-regulation of Ruk/CIN85 (generated early using lentiviral technology) were used as a model for research. Melanoma cells were cultured in the complete RPMI 1610 medium under standard conditions. Proliferative activity of the cells was estimated using the MTT-test, and cell migratory potential was studied by the wound-healing assay. The data obtained were analyzed with parametric Student`s t-test. Results were expressed as mean ± SEM and significance was set at $P < 0.05$.

Results and Discussion. Cutaneous melanoma genesis is a multi-step process initiated by the transformation of a normal melanocyte following an oncogenic insult. Due to the transcriptome and metabolome reprogramming in the course of EMT, transformed melanoma cells change their phenotype and acquire increased proliferative rate, cell motility, invasiveness, and metastatic potential. According to the data obtained, overexpression of Ruk/CIN85 in B16 mouse melanoma cells (subclones Up7 and Up21) led to an increase in their proliferative activity by 1,6 and 1,8 times, respectively, at 24th hour in comparison with control Mock cells (Fig. *A*). At the 48th hour, when the cells reached confluence, the cell viability of subclones did not differ from the control ones. No statistically significant changes in the proliferative activity of B16 cells with suppressed expression of the adaptor protein (subclone Down) were found. In accordance with previous data [4], B16 cells overexpressing Ruk/CIN85 were characterized by strongly increased motility rate (more than twofold for both Up7 and Up21 subclones compared to control Mock cells). At the same time, knockdown of Ruk/CIN85 in B16 cells resulted in a decrease in their migratory activity by about 30% (Fig. *B*).

Conclusions. All findings obtained demonstrated that the malignancy traits of melanoma B16 cells are inversely modulated upon up- and down-changes in adaptor protein Ruk/CIN85 expression levels suggesting its possible role in the control of EMT.

Key words: melanoma, adaptor proteins, Ruk/CIN85, epithelial-mesenchymal transition, proliferation, migration.

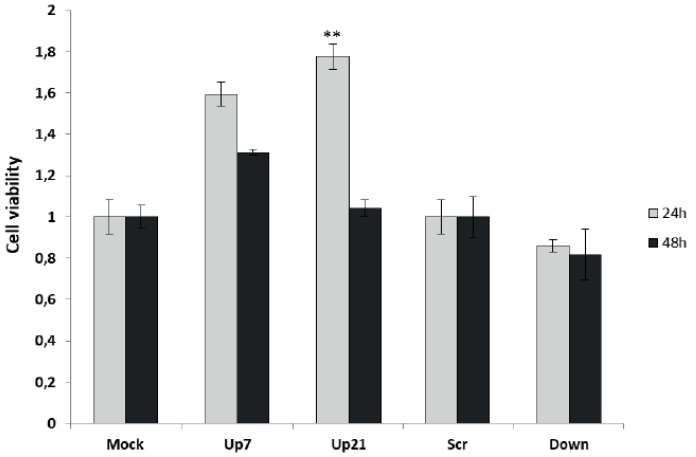

Fig. **Adaptor protein Ruk/CIN85 modulates viability and motility rate of mouse melanoma B16 cells depending on its expression level:**
A — cell viability; *B* — motility rate. (Up7, Up21 – subclones of B16 cells verexpressing Ruk/CIN85 and corresponding control Mock cells; Down – B16 cells with down-regulation of Ruk/CIN85 and corresponding control Scr cells).
$M \pm m$, $\boldsymbol{n} = 3$, *,** $P < 0.05$ to Mock and Scr.

The authors state that they have no conflict of interest.

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