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DEFICIENCY OF ADAPTOR PROTEIN RuK/CIN85 IN LEWIS LUNG CARCINOMA CELLS INHIBITS MALIGNANCY HALLMARKS *IN VITRO* AS WELL AS TUMOR GROWTH AND PULMONARY METASTASIS *IN VIVO*

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Introduction. Lung cancer is a common cause of cancer mortality associated with distant metastases. Progress in metastatic research, however, is constrained by the lack of tumor-bearing animal models that would allow understanding comprehensively the complex network of signaling pathways that drives the multistep process of metastatic cascade. Adaptor/scaffold proteins are key regulators able to effectively process the information through signaling networks. It was shown previously that high levels of adaptor protein Ruk/CIN85 contribute to the conversion of weakly invasive human breast adenocarcinoma MCF-7 cells into a more malignant phenotype. In addition, we found high amounts of this adaptor in aggressive Lewis lung carcinoma cells (LLC cells). In the current study, we set a goal to determine interplay between Ruk/CIN85 down-regulation in LLC cells and their metastatic potential using experimental and spontaneous metastasis models in syngeneic C57BL/6 mice.

Methods. To down-regulate Ruk/CIN85, LLC cells were stably infected with lentivirus encoding Ruk/CIN85-specific shRNA as well as irrelevant virus to obtain control cells. The expression levels of Ruk/CIN85 in LLC cells and primary tumors were assessed by Western-blotting. Cancer cells proliferation was studied by direct cell count and MTT test, cell migration – by scratch test and invasiveness – using Boyden chamber assay. The influence of Ruk/CIN85 down-regulation on the morphology of LLC cells was studied by confocal microscopy. The expression levels of specific EMT markers were evaluated by qRT-PCR. To estimate efficiency of experimental and spontaneous metastasis, C57BL/6 mice were inoculated intravenously or subcutaneously into right hind leg with control and Ruk/CIN85 down-regulated LLC cells. Primary tumors and lungs were processed for histological evaluation according to standard protocol. Statistical analysis was carried out using ANOVA with Newman-Keuls correction.

Results. It was demonstrated that Ruk/CIN85 knockdown in LLC cells caused attenuation of their proliferative rate, decreased motility and invasiveness while increased adhesion properties *in vitro*. Down-regulation of Ruk/CIN85 significantly reduced metastatic potential of LLC cells both in experimental and spontaneous metastasis models *in vivo*. According to changes in cell morphology and qRT-PCR data, the suppression of aggressiveness of Ruk/CIN85 knockdown cells was associated with mesenchymal-to-epithelial transition.

Conclusions. Taken together, the data obtained suggest that adaptor protein Ruk/CIN85 could function as a concentration-dependent switch of mesenchymal-to-epithelial transition in Lewis lung carcinoma cells being thus a promising target for therapeutic intervention.

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