

## THE ACQUISITION OF RESISTANCE IN HUMAN NON-SMALL LUNG ADENOCARCINOMA MOR CELLS IS ASSOCIATED WITH UP-REGULATION OF ADAPTOR PROTEIN RUK/CIN85 AND EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT)

Y. Raynich<sup>2</sup>, T. D. Skaterna<sup>1</sup>, D. S. Gerashenko<sup>1</sup>, O.V. Khudiakova<sup>1</sup>,  
L. V. Garmanchuk<sup>1</sup>, L. B. Drobot<sup>1</sup>

<sup>1</sup>Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv  
<sup>2</sup>Taras Shevchenko National University, Kyiv, Ukraine

*E-mail: yanaraynich@gmail.com*

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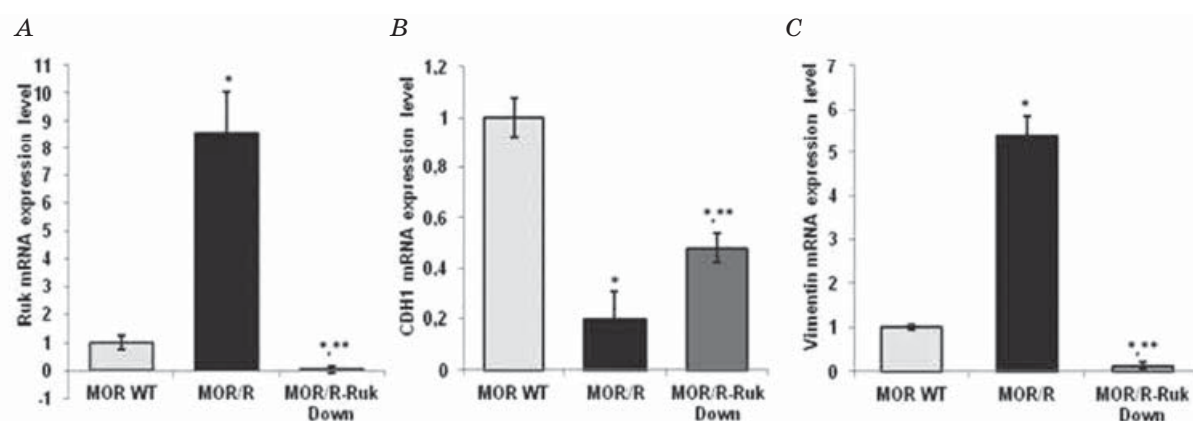
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Non-small-cell lung carcinoma (NSCLC) is the most common type of cancer and cancer-related death in the world. High resistance of NSCLC to traditional chemotherapy is a serious problem for the effectiveness of treatment. Ruk/CIN85 is an adaptor/scaffold protein involved in the processes of tumor cells malignant transformation. By modulating protein-protein interactions in space and time, it potentially regulates signaling networks that control EMT-related cell responses such as proliferation, migration, invasiveness, and metastasis in the course of cancer progression [1].

*The aim* of this study was to elucidate the regulatory role of Ruk/CIN85 in chemoresistance and EMT using human NSCLC MOR cells as a model [2].

*Methods.* MOR (ECACC 84112312) cell line and drug-resistant cell line MOR/0.2R (ECACC 96042335) were cultured under standard conditions in DMEM medium. Knockdown of Ruk/CIN85 in MOR/0.2R cells was performed using shRNA lentiviral technology. Expression levels of Ruk/CIN85, vimentin and E-cadherin were estimated by RT-PCR.



**Fig. Knockdown of Ruk/CIN85 in NSCLC MOR doxorubicin-resistant cells induces mesenchymal-epithelial transition:**

A, B, C — relative Ruk/CIN85, CDH1 and vimentin expression levels, respectively. (MOR WT — parental cells; MOR/R — drug-resistant MOR cells; MOR/R-Ruk Down — resistant MOR cells with Ruk/CIN85 knockdown);  $M \pm m$ ,  $n = 3$ , \*  $P < 0.05$  to MOR WT, \*\*  $P < 0.05$  to MOR/R

**Results and Discussion.** Taking into account the available data that up-regulation of Ruk/CIN85 in breast adenocarcinoma cells is followed by increase in their chemoresistance [3], we first compared the adaptor protein expression levels in MOR and MOR/0.2R cells (doxorubicin selected cells). According to the results of qPCR, MOR/0.2R cells showed an extremely higher level of Ruk/CIN85 mRNA expression, more than 10 times higher than the parental MOR cells (Fig. A). These results were supported by data of Western-blotting. Also, preliminary data obtained in the Department of Cell Signaling revealed that knockdown of Ruk/CIN85 in the MOR/0.2R cells led to significant decrease of their resistance to doxorubicin and development of epithelial phenotype. So, in order to study the role of Ruk/CIN85 in EMT, we decided to check the expression levels of EMT epithelial marker E-cadherin as well as mesenchymal marker vimentin [4] in MOR sublines depending on adaptor protein expression levels. As can be seen from Figure, high content of Ruk/CIN85 in doxorubicin-resistant (MOR/R) cells strongly correlate with their mesenchymal phenotype (high expression level of vimentin and low — E-cadherin), while its down-regulation is followed by restoration of expression values characteristic of parental MOR cells.

**Conclusions.** In summary, high expression level of Ruk/CIN85 in doxorubicin-resistant MOR cells and the reversion of EMT-related transcriptome parameters and sensitivity to drug due to knockdown of adaptor protein in this subline suggests its involvement in regulation of EMT as well as cancer cells chemoresistance. Thus, the adaptor protein Ruk/CIN85 can be considered as a tissue-specific marker of carcinogenesis and perspective target for drug development.

**Key words:** NSCLC, adaptor proteins, Ruk/CIN85, chemoresistance, epithelial-mesenchymal transition.

The authors state that they have no conflict of interest.

#### REFERENCES

1. Havrylov S., Redowicz M. J., Buchman V. L. Emerging roles of Ruk/CIN85 in vesicle-mediated transport, adhesion, migration and malignancy. *Traffic*. 2010, 11(6), 721–731. <https://doi.org/10.1111/j.1600-0854.2010.01061.x>.
2. Twentyman P. R., Fox N. E., Wright K. A., Bleehen N. M. Derivation and preliminary characterisation of adriamycin resistant lines of human lung cancer cells. *Br. J. Cancer*. 1986; 53(4):529–37. <https://doi.org/10.1038/bjc.1986.83>.
3. Horak I. R., Geraschenko D. S., Drobot L. B. Adaptor protein Ruk/CIN85 modulates resistance to doxorubicin of murine 4T1 breast cancer cells. *Ukr. Biochem. J.* 2018; 90(3): 94–100. <https://doi.org/10.15407/ubj90.03.094>
4. Lamouille S., Xu J., Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. *Nat. Rev. Mol. Cell. Biol.* 2014; 15(3): 178–196. <https://doi.org/10.1038/nrm3758>.