UDC 577.112

ADAPTOR PROTEIN Ruk/CIN85 IS A NOVEL MOLECULAR COMPONENT OF EXTRACELLULAR VESICLES PRODUCED BY TUMOR CELLS

A. Y. ZHYVOLOZHNYI[1], *M. S. KIRYAKULOVA*[1], *A. A. SAMOYLENKO*[2], *S. VAINIO*[2], *L. B. DROBOT*[1]

[1]*Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;*
[2]*Laboratory of Developmental Biology, Biocentre Oulu, Faculty of Biochemistry and Molecular Medicine, University of Oulu, Oulu, Finland;*
e-mail: ppndl2@gmail.com

Introduction. Nanosized membrane vesicles, termed "exosomes", are secreted by most cell types under both physiological and pathological conditions, especially by tumor cells, and were proposed to be actively involved in intercellular signaling (Yang & Robbins, 2011). There are data that proteins Alix and Tsg101 (Schmidt et al., 2005; Segura-Morales et al., 2005) involved in the formation of MVB (multivesicular bodies) and currently recognized as marker proteins of exosomes, as well as cortactin, which stimulates the secretion of exosomes (Lynch et al., 2003; Sinha et al., 2016), are the binding partners of adaptor protein Ruk/CIN85. In addition, we have shown previously that up-regulation of the adaptor protein Ruk/CIN85 in breast cancer cells is involved in the stabilization of the transcription factor HIF1α (Samoylenko et al., 2010). Taking into account these data, the main aim of our study was to elucidate the role of Ruk/CIN85 in biogenesis of extracellular membrane vesicles (EVs) produced by tumor cells and assess the influence of hypoxia conditions on this process.

Methods. Renca cells (mouse renal cell carcinoma) were cultured under standard conditions. The hypoxic environment was created by incubating the cells in a standard 5% CO_2 incubator infused with N2 to create a constant 1% O_2 environment. Normoxia was defined as 21% O_2 environment supplemented with 10% CO_2. To obtain Ruk/CIN85-overexpressing cells, Renca cells were transfected with pRc/CMV2-Rukl plasmid encoding the full-length form of Ruk/CIN85 or empty vector using Lipofectamine 2000 reagent followed by selection of stable transfectants in the presence of Geneticin. EVs were isolated by concentration of conditioned medium with Centricon Plus-70 followed by ultracentrifugation at 100 000 g. The number and size of EVs were assessed using NanoSight device and morphology – by electron microscopy. The protein content of EVs was studied by Western-blot analysis.

Results. To achieve the main aim of our work, we created subline of Renca cells stably overexpressing the adaptor protein Ruk/CIN85. Using Western-blot analysis of whole cell lysates, it was demonstrated that up-regulation of Ruk/CIN85 in Renca cells results in a significant increase in the expression level of Alix protein. Importantly, Ruk/CIN85 and Alix expression levels were decreased under hypoxic conditions in both control and Ruk/CIN85-overexpressing cells. Higher content of Ruk/CIN85, concomitantly with marker proteins Alix and CD81, was detected in EVs preparations isolated from conditioned medium of Ruk/CIN85-overexpressing cells in comparison with control ones. Under hypoxic conditions, increased levels of Ruk/CIN85 and CD81 were observed in EVs produced by control cells while decreased levels - in EVs produced by Ruk/CIN85-overexpressing cells. At the same time, hypoxia caused decrease of Alix protein content in EVs from both cell types.

Conclusions. It was demonstrated that the adaptor protein Ruk/CIN85 is a newly identified component of exosomes produced by tumor cells. The potential role of Ruk/CIN85 in the control of protein composition of exosomes under conditions of normoxia and hypoxia was established.

Acknowledgments. This study was supported by CIMO Fellowships Grant (Finland).