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COMPREHENSIVE OVERVIEW OF ADAPTOR PROTEIN RUK/CIN85 ROLES IN CANCER

I. HORAK[1, 2], *T. SKATERNA*[1], *O. HUDKOVA*[1], *N. LATYSHKO*[1], *O. KHUDIAKOVA*[1], *K. TOKARCHUK*[1], *I. KRYSIUK*[1], *T. KISHKO*[1], *L. KNOPFOVÁ L. DROBOT*[1]

[1]Department of the Cell Signaling,
Palladin Institute of Biochemistry of National Academy of Sciences (NASU), Kyiv, Ukraine
[2]Laboratory of Cell Differentiation, Department of Sciences, Masaryk University,
Brno, the Czech Republic

E-mail: iryna.horak@gmail.com

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The novel edition of "Hallmarks of Cancer" incorporated additional emerging hallmarks and enabling characteristics including "unlocking phenotypic plasticity" [1]. Cancer cell plasticity refers to the remarkable ability of cancer cells to adapt, change, and survive in diverse environments, contributing to tumor heterogeneity and therapeutic resistance. This phenomenon is a major challenge in cancer treatment and underscores the complexity of the disease.

Aim. This study is focused on a comprehensive overview of mechanisms and processes involved in the acquisition of cancer cell plasticity in a manner dependent on the adapter protein Ruk/CIN85 (in rodents, Ruk — regulator of ubiquitous kinase; in human CIN85 — Cbl-interacting protein of 85 kDa, encoded by *SH3KBP1* gene). This adaptor protein was found to be overexpressed in various cancers compared to normal tissue, including breast [2], prostate cancer [3], esophageal squamous cell carcinoma [4], head and neck squamous cell carcinoma [5], and colon adenocarcinoma [6], moreover, its expression correlates with metastases and advanced clinical stage.

Methods. Breast (MCF-7, 4T1), lung (LLC, A549) cancer, osteosarcoma (HOS, SAOS-2) cells with Ruk/CIN85 overexpression and/or knockdown, as well as corresponding controls were used in the experiments. Gene expression was evaluated using RT^2-PCR and Western blotting, cell proliferation and survival were analyzed using MTT and/or dye exclusion assays, motility was assessed by scratch test and Transwell assay, enzyme activities were measured using spectrophotometric assays. *In vivo* metastasis were studies using experimental metastasis model.

Results. One of the main and well-studied mechanisms of cancer cell plasticity is the epithelial-mesenchymal transition (EMT), which allows cancer cells to become more motile, invasive, and resistant to apoptosis. EMT is driven by various signaling pathways, including TGF-β, Wnt, and Notch, as well as transcription factors Snail, Zeb, and Twist. Conversely, mesenchymal-epithelial transition (MET) enables mesenchymal-like cancer cells to revert to an epithelial phenotype. MET is crucial for the colonization of distant organs during metastasis, as epithelial traits promote cell-cell adhesion and proliferation in secondary sites. The balance between EMT and MET is tightly regulated and influences cancer cell behavior at different stages of disease progression.

Cancer stem cells (CSCs) also contribute to plasticity by exhibiting self-renewal and differentiation capacities, as well as anticancer drug resistance. CSCs can generate heterogeneous cell populations within tumors, driving tumor growth and therapy resistance. Signaling pathways such as Notch, Hedgehog, and Wnt regulate CSC properties, highlighting their role in tumor plasticity.

Additionally, the tumor microenvironment (TME) plays a critical role in modulating cancer cell plasticity. Interactions with stromal cells, immune cells, and extracellular matrix components influence cancer cell phenotypes. TME factors such as hypoxia, inflammation, and nutrient availability can induce phenotypic changes in cancer cells, promoting survival and metastasis.

Therapeutic strategies targeting cancer cell plasticity are actively being explored. Inhibitors of EMT-inducing pathways, CSC-specific markers, and TME components are under development to disrupt plasticity-driven mechanisms. Combination therapies that target multiple aspects of plasticity may improve treatment outcomes and overcome therapy resistance.

Using cellular models of breast, lung, and bone cancer, the effects of Ruk/CIN85 on multiple attributes of cancer cell plasticity were investigated. It was demonstrated that overexpression of Ruk/CIN85 resulted in elevated motility in vitro and metastatic potential in vivo, while Ruk/CIN85-knockdown cells are characterized by a lack of migration and metastasis. In addition, a high expression level of Ruk/CIN85 is necessary for the acquisition of resistance to anticancer drugs. Analysis of possible molecular mechanisms revealed expression of EMT- and CSCs-related markers, ECM remodeling ability, as well as metabolic reprogramming (Warburg effect) in Ruk/CIN85-overexpressing cells. On the contrary, Ruk/CIN85 knockdown resulted in the acquisition of a more differentiated phenotype with increased adhesion and proliferation. These findings are summarized in the Figure.

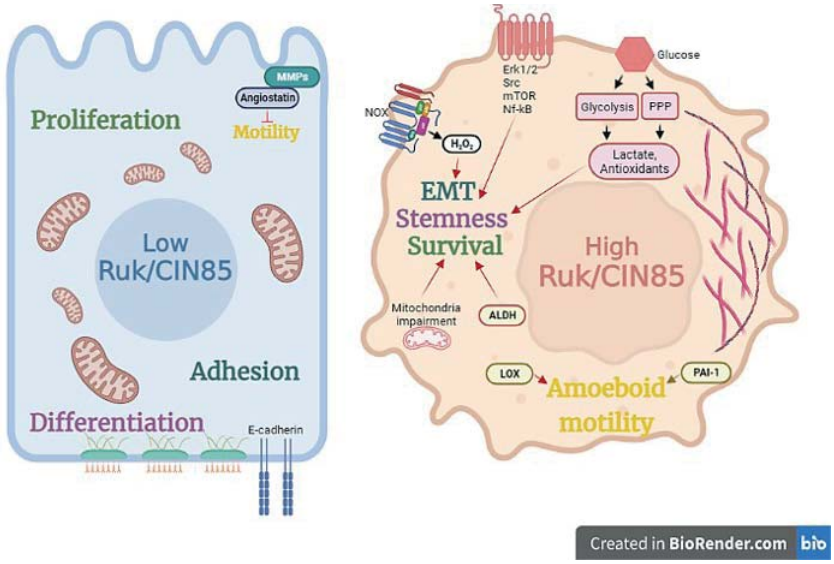

Fig. **Effect of adaptor protein Ruk/CIN85 on cancer cell plasticity-related features**

Conclusion. Taken together, this study discloses various aspects of cancer cells plasticity, such as EMT, stemness, metabolic changes, ECM components, and drug resistance in dependence on adaptor protein Ruk/CIN85 expression level.

Key words: adaptor protein Ruk/CIN85, breast cancer, lung cancer, osteosarcoma, motility, metastasis, cancer stem cells, drug resistance, cancer cell plasticity.

Authors' contribution. IH and TS performed motility and drug resistance assays, gene expression analysis, OH, NL, KT, IK, and TK analyzed metabolic parameters, IH and LB curated research planning, data analysis, and manuscript writing.

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