UDC 557.112 + 577.121 https://doi.org/10.15407/biotech15.02.047

ADAPTOR PROTEIN RUK/CIN85 IS INVOLVED IN THE GLUCOSE METABOLISM REPROGRAMMING IN BREAST CANCER CELLS

M. I. Bekala[1, 2], I. R. Horak[1,3], O. O. Gudkova[1], N. V. Latyshko[1], K. O. Tokarchuk[1], D. S. Geraschenko[1], M. O. Shkoda[2], L. B. Drobot[1]

[1] Palladin Institute of biochemistry of the National Academy of Sciences of Ukraine, Kyiv
[2] Taras Shevchenko National University of Kyiv, Kyiv
[3] Masaryk University, Brno, Czech Republic

E-mail: markbekala2@gmail.com

Receivd 19.04.2022
Revised 27.05.2022
Accepted 30.05.2022

To survive under hypoxia and lack of nutrients and to efficiently metastasize, cancer cells are able to modify their metabolism. Moreover, cancer stem cells (CSCs) were reported to have elevated glycolysis and altered glutamine and fatty acids metabolism [1]. Our previous findings demonstrated that overexpression of adaptor protein Ruk/CIN85 in breast cancer cells led to increased motility, invasiveness, and the manifestation of the CSCs features [2, 3].

Aim. This study aimed to investigate the changes in glucose metabolism in mouse 4T1 breast adenocarcinoma cells with different levels of Ruk/CIN85 expression.

Methods. We used 4T1 cells with stable overexpression (subline RukUp) or knockdown (subline RukDown) of Ruk/CIN85, as well as corresponding vector control sublines Mock and Scr. Cells were cultured in the complete RPMI-1640 medium under standard conditions. mRNA expression levels were estimated by RT^2-PCR, enzymes activities were measured by spectrophotometric and/or fluorometric assays.

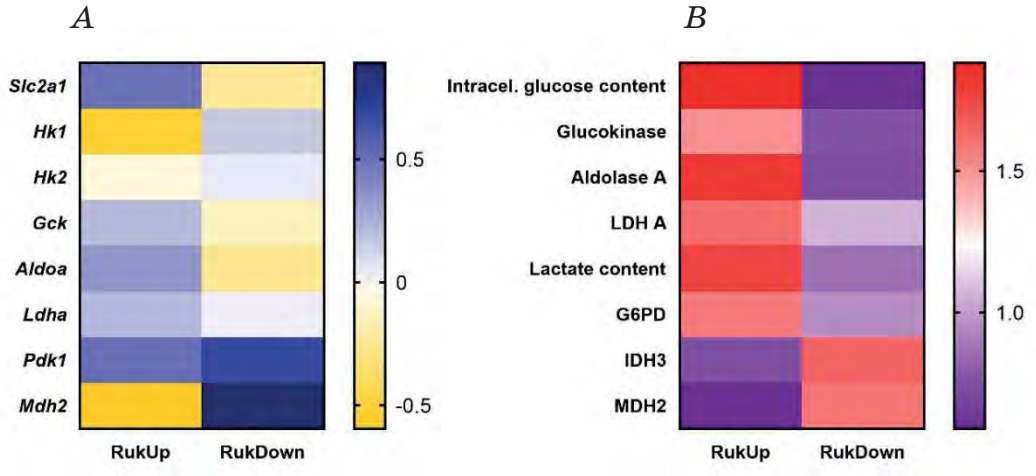

Fig. **Heatmap representation of gene expression (*A*) and enzyme activities or metabolites content (*B*) in 4T1 cells with overexpression and downregulation of the adaptor protein Ruk/CIN85 (*GraphPad Prism*)**

Results. Analysis of mRNA expression of glucose metabolism-related genes in RukUp and RukDown cells revealed that glycolysis genes are preferentially overexpressed in RukUp cells, and downregulated in RukDown cells (Fig. *A*). Thus, RukUp cells were characterized by significantly overexpressed *Slc2a1, Gck, Aldoa,* and *Ldha,* while in RukDown cells these genes were either down regulated or not changed. However, the expression of TCA (tricarboxylic acid) cycle enzyme *Mdh2* increased dramatically (by 7,8 times) in RukDown cells. These findings were confirmed and complemented by enzyme activities and metabolites analysis. Fig. *B* clearly indicates that high level of Ruk/CIN85 is strongly associated with elevated glycolysis, and low level of Ruk/CIN85 — with TCA and mitochondrial oxidation. In detail, we observed statistically significant changes in the activity of all studied enzymes in RukUp cells (increase by 1.5–1.9 times for glycolysis enzymes and G6PD, and decrease by 1.33–1.69 times for TCA enzymes). However, in RukDown cells we did not find any significant changes in glycolysis enzymes activities, but activities of mitochondrial IDH3 and MDH2 were elevated by 1.65 and 1.59 times, respectively.

Discussion. In this study we found that high expression level of Ruk/CIN85 is strongly associated with elevated glucose uptake, increased glycolysis, and diminished mitochondrial functioning. These features are characteristic of various highly aggressive malignant tumors and are known as the Warburg effect. Elevated glucose oxidation via glycolysis provides plenty advantages for cancer cells, such as fast ATP production, essential metabolic intermediates biosynthesis, avoiding oxidative stress, etc, that allows us to consider glycolysis is a promising target for anti-cancer therapy [4]. In addition, Ruk/CIN85 knockdown resulted in decreased glucose uptake and its elevated oxidation in the TCA cycle which is characteristic for normal epithelial cells.

Conclusions. The results obtained indicate that adaptor protein Ruk/CIN85 is involved in the metabolic reprogramming during breast cancer progression. High level of Ruk/CIN85 expression is associated with potentiation of the Warburg effect.

Key words: breast cancer, the Warburg effect, adaptor proteins, Ruk/CIN85, metabolic reprogramming.

Funding source. This research was partially supported by the NRFU grant 2020.02/0195 “Differential control of regulatory networks involved in the maintaining of epithelial-mesenchymal plasticity of breast adenocarcinoma cells”.

REFERENCES

1. *Mukha A., Dubrovska A.* Metabolic Targeting of Cancer Stem Cells. *Front Oncol*. 2020; 10: 537930. https://doi.org/10.3389/fonc.2020.537930
2. *Horak I. R., Pasichnyk G. V., Gerashchenko D. S, Knopfova L., Borsig L., Drobot L. B.* Adaptor protein Ruk/CIN85 modulates manifestation of cancer stem cells (CSCs) features in mouse breast adenocarcinoma 4T1 cells. *Reports of the National Academy of Sciences of Ukraine*. 2018; 12: 101–109. https://doi.org/10.15407/dopovidi2018.12.101
3. *Horak I., Drobot L., Borsig L., Knopfova L., Smarda J.* Overexpression of adaptor protein Ruk/CIN85 in mouse breast adenocarcinoma 4T1 cells induces an increased migration rate and invasion potential. *Biopolymers and Cell*. 2018; 34 (4): 284–291. http://dx.doi.org/10.7124/bc.000981
4. *Abdel-Wahab A. F., Mahmoud W., Al-Harizy R. M*. Targeting glucose metabolism to suppress cancer progression: prospective of anti-glycolytic cancer therapy. *Pharmacol Res*. 2019; 150: 104511. https://doi.org/10.1016/j.phrs.2019.104511