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ADAPTOR PROTEIN RUK/CIN85 PARTICIPATES IN THE METABOLIC CONTROL OF HUMAN BREAST ADENOCARCINOMA MCF-7 CELLS

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Cancer cells are able to dynamically change their metabolism to promote survival, maintain proliferative activity, provide high migration and invasive potential and increase metastasis. A common feature of this altered metabolism is increased glucose uptake and its fermentation to lactate, a phenomenon known as the Warburg effect [1].

Aim. To determine the role of Ruk/CIN85 in the control of breast adenocarcinoma cells metabolism, we performed systemic analysis of the activity levels/content of key enzymes/components of glycolysis and oxidative phosphorylation using as a model the weakly invasive human breast adenocarcinoma MCF-7 cell line (Mock); and its sublines with stable overexpression (G4 subline) and reverse down-regulation (G4vir subline) of the adaptor protein [2].

Materials and Methods. MCF-7 cells were cultured in the complete DMEM medium under standard conditions. Enzymes activity, content of metabolites and protein in cell extracts and the conditioned cell culture medium were estimated by spectrophotometric and fluorometric assays. The data obtained were analyzed with parametric Student`s t-test. Results were expressed as mean ± SEM and significance was set at $P < 0.05$.

Results and Discussion. First of all, biochemical indexes of aerobic glycolysis, activity levels of some key glycolytic enzymes and metabolites were evaluated [3]. A significant increase in the activity of these enzymes, aldolase A (ALDOA) and lactate dehydrogenase A (LDHA), was found in G4 cells compared to Mock by 1.3 and 1.6 times, respectively (Fig. *A*, *B*). In addition, in the conditioned medium of G4 cells, an increase in lactate content by 1.5 times compared with the control was found, which corresponded to a change in LDHA activity (Fig. *C*). Knockdown of Ruk/CIN85 expression level in G4 subline resulted in a significant decrease of these parameters compared to G4 cells, ALDOA — 4 times, LDHA — 1.4 times, and lactate production — 2.5 times. It should be noted that in G4vir cells, LDHA activity returned to level of control cells, while ALDOA activity and lactate content additionally decreased by 3 times and 1.6 times, respectively. Therefore, the observed changes in the intensity of glycolysis in MCF-7 sublines positively correlate with the expression level of adaptor protein studied.

To assess the metabolic status of mitochondria, the level of activity of the Krebs cycle enzyme, NAD-dependent malate dehydrogenase (MDH2), the catalyst of last stage of the cycle, was determined [3]. A 2-fold decrease in MDH2 activity was found in the MCF-7 G4 subline relative to control Mock cells, as well as an increase in this index by 2.4 times in G4vir cells to control values (Fig. *D*). Unlike glycolysis, we observed the opposite pattern with respect to the intensity of Krebs cycle reactions depending on the expression level of Ruk/CIN85.

Conclusions. The observed reversion of the Warburg metabotype as a result of Ruk/CIN85 down-regulation in MCF-7 cells overexpressing adaptor protein is a strong experimental evidence for its regulatory role in energy supply modes, aerobic glycolysis versus OXPHOS in the course cancer cells malignization.

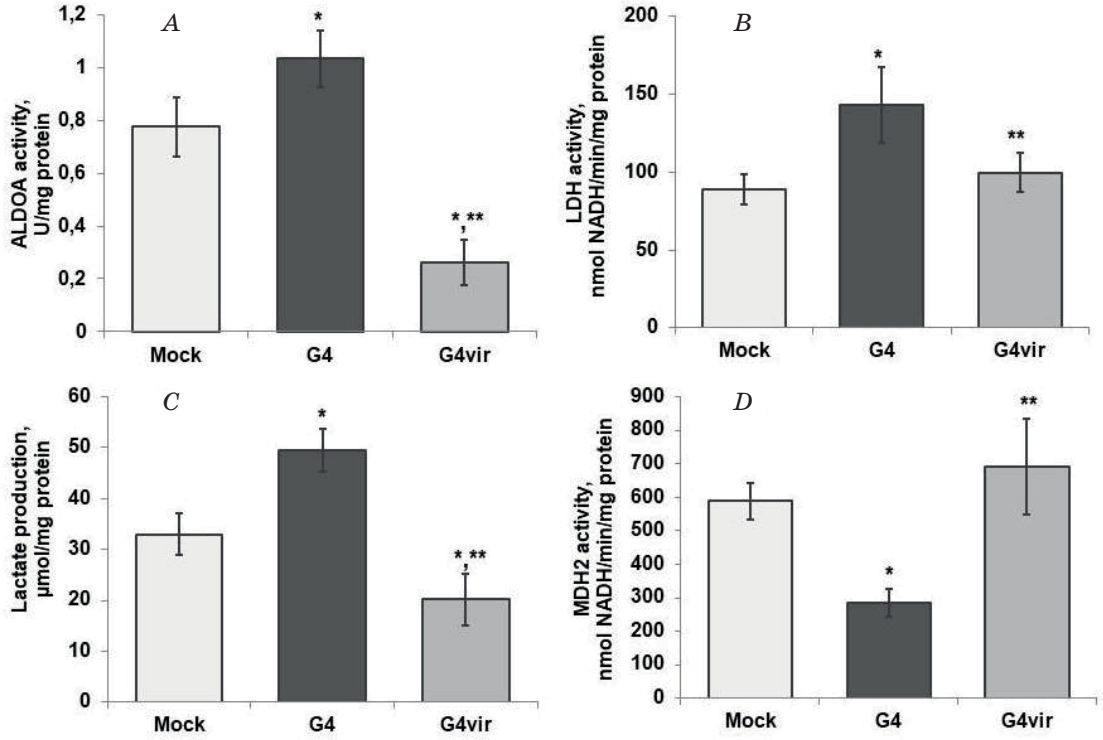

Fig. **Adaptor protein Ruk/CIN85 modulates metabolic indexes associated with Warburg effect in MCF-7 cells depending on its expression level**

Activities of enzymes: ALDOA (*A*), LDHA (*B*), MDH2 (*D*) in the cell extracts and lactate content in the conditioned cell culture medium (*C*), $M \pm m$, $n = 3$, * $P < 0.05$ to Mock, ** $P < 0.05$ to G4.

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Key words: breast adenocarcinoma cells, adaptor protein, Ruk/CIN85 Warburg effect, aerobic glycolysis, oxidative phosphorylation, aldolase A, lactate dehydrogenase A, lactate, NAD-dependent malate dehydrogenase.

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