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Valproic acid (VPA) exerts different effects on proliferation and viability of 4T1 parental and 4T1 cells overexpressing adaptor protein Ruk/CIN85 by triggering metabolic reprograming in type specific manner

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Background/Aim. We previously demonstrated that mouse breast adenocarcinoma 4T1 cells overexpressing adaptor protein Ruk/CIN85 (4T1 RukUp) acquired a malignant phenotype associated with cancer stem cells features compared with parental 4T1 cells (4T1 Mock) [1]. VPA, a broad Class I histone deacetylases inhibitor, is widely used in cancer therapy [2]. The study aimed was to evaluate the effect of VPA on Warburg effect dependent on Ruk/CIN85 expression in 4T1 cells. **Methods.** The methods of cell culturing, microscopy, spectrophotometry, and flow cytometry were used [3, 4]. Cell viability/metabolic activity was analysed using the MTT assay in the VPA concentration range of 1–6 mM at the 48th hour of cell culturing. **Results and Discussions.** 3 mM VPA enhanced the epithelial morphology of 4T1 Mock cells while induced a transition from a mesenchymal/amoeboid to an epithelial/mesenchymal phenotype in 4T1 RukUp cells. Under these conditions, the proliferative activity of Mock cells was inhibited by 1.69 and RukUp cells by 1.95 times. The metabolic activity of 4T1 Mock cells was dose-dependently decreased in the presence of VPA and was unchanged in 4T1 RukUp cells. VPA inhibited aerobic glycolysis by decreasing the activities of key glycolytic enzymes (hexokinase, aldolase, and lactate dehydrogenase) and lactate content in the conditioned medium by 20–30% compared with corresponding controls. The value of mitochondrial membrane potential in RukUp cells was about 3 times lower compared to Mock cells, which is characteristic of highly malignant cancer cells [5]. Treatment of cells with 3 mM VPA resulted in an increase in this parameter (Mock cells — 1.65-fold; RukUp cells — 2.25-fold), which was reversed in direction compared to the activity of glycolytic enzymes. **Conclusions.** The results obtained demonstrate that VPA restores the coupling between oxidative phosphorylation and glycolysis in 4T1 RukUp cells indicating a novel mechanism of its anticancer effects. **Grants/Fundings.** The present study was granted by the National Research Foundation of Ukraine (project N 2020.02/0195).

K e y w o r d s: carcinogenesis, 4T1 cells, Ruk/CIN85, VPA.

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