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CRISPR/Cas9 – mediated gene silencing of OXCT1 in HeLa cells favors cell proliferation

Marta Biesiekierska[1]✉, Maciej Studzian[1], Paweł Hikisz[1], Souad Nasser[2], Luciano Pirola[2] and Aneta Balcerczyk[1]

[1]University of Lodz, Faculty of Biology and Environmental Protection, Department of Molecular Biophysics, Lodz, Poland; [2]INSERM Unit 1060, CarMeN Laboratory, Lyon 1 University, France;
✉***Corresponding author, E-mail:*** ***marta.biesiekierska@edu.uni.lodz.pl.***

Abstract

Extensive evidence suggests that the utilization of ketone bodies as an alternative source of energy to carbohydrates may have a beneficial effect on cancer treatment due to the fact that the major ketone body – β-hydroxybutyrate – directly affects inflammatory processes. The rate-limiting enzyme in ketone bodies catabolism is succinyl-CoA:3-ketoacid-coenzyme A transferase 1 (SCOT1), encoded by the OXCT1 gene. Our previous studies showed an elevated OXCT1 expression in various cancer cell lines, including cervical cancer cell line HeLa. The main aim of the research was to knockout the OXCT1 gene from HeLa cells using the CRISPR/Cas9 technique in order to analyze the proliferation rate and possible new functional characteristics of this model.

The knocking-out procedure of OXCT1 was performed by CRISPR/Cas9 and gDNA transient transfection, followed by a Surveyor test to scan for the occurrence of the DNA mutation. The SCOT1 protein ablation in multiple clones was determined by Western blotting and immunofluorescent microscopy. Moreover, we performed a cytometric analysis of the cell cycle phase distribution and analyzed the gene expression of selected cell cycle determinants, including p21 and cyclins.

We observed that OXCT1 knockout increases the proliferation rate of HeLa cells, suggesting that OXCT1 gene may be non-essential for cell proliferation or even involved in attenuation of cell proliferation, suggesting that a switch to an alternative energy source to glucose/carbohydrates in cancer cells may render them less susceptible to proliferation.

Keywords: cancer cells, ketone bodies, metabolism

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