Секция «Биоинженерия»

The Role of ThDP-dependent Enzymes in Viability of Glioblastoma Cells.

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The thiamine diphosphate (ThDP)-dependent dehydrogenases of 2-oxoglutarate (2-OGDH), pyruvate and branched chain 2-oxo acids were shown to significantly contribute to mitochondrial ROS production in the condition-dependent manner, with the highest maximal capacity to produce ROS attributed to 2-OGDH [1,4]. Nevertheless, 2-OGDH inhibition either with specific inhibitors or with genetic manipulation may enhance ROS production, decreasing cellular viability [3]. The data obtained suggest that impaired metabolism induces a perturbation-specific ROS sources *in vivo*. To assess the significance of ThDP-dependent enzymes for the viability of glioblastoma cells we compared the effect of thiamine and its antagonist oxythiamine on the physiology of U87 and T98G cell lines *in situ*. ATP, NAD(P)H (reductive ability) and ROS levels were estimated using CellTiter-Glo, CellTiter-Blue viability assays and DCFDA Cellular ROS Detection Assay respectively. The influence of oxythiamine on ThDP-dependent enzymes in cells after incubation was confirmed by inhibition of 2-OGDH, which has the strongest affinity to ThDP among mammalian ThDP-dependent enzymes [2]. The time and concentration-dependent effects on NAD(P)H and ROS were significantly different for thiamine and oxythiamine, but little effect on ATP level was observed. Surprisingly, oxythiamine caused an increase in cellular NAD(P)H level concomitant with a strong decrease in cellular ROS. In contrast, reducing potential was not increased by thiamine, causing even stronger decrease in ROS compared to the effect of oxythiamine. Thus, both thiamine and oxythiamine have antioxidant properties. However, the oxythiamine-impaired metabolism corresponds to a redox state where cells exhibit increased levels of both NAD(P)H and ROS, compared to the redox state established in the presence of thiamine.

Источники и литература

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Слова благодарности

The author thanks Frank Gaunitz and Henry Oppermann for hospitality and help and his tutor Victoria Bunik for trust and thoroughness.