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A novel prognostic marker with metabolic implications in anaplastic glioma: a carnitine transporter and its role in treatment resistance

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The diagnosis of malignant glioma afflicts approximately 6 persons in 100,000 people per year and, despite active research in the last years, is still associated with a dismal prognosis. When epigenetic silencing of the O6-methylguanine-DNA-methyltransferase (MGMT) promoter was associated with increased sensitivity to temozolomide, a predictor of improved outcome after alkylating chemotherapy in patients with newly diagnosed glioblastoma was found. Unexpectedly, the NOA-04 trial on patients with WHO grade III anaplastic gliomas showed a better patient outcome of MGMT hypermethylated patients not only in the chemotherapeutic arm, but also in the radiotherapy-only arm. These findings support the assumption of a more complex methylation phenotype of prognostic value also for WHO grade III anaplastic glioma, as it has been recently described for glioblastoma in the glioma CpG island methylation phenotype (G-CIMP). The NOA-04 glioma samples were therefore screened for candidate genes that were co-methylated with MGMT and had a prognostic impact on outcome after radio- or chemotherapy. Possible candidates were validated on two independent glioma collectives.

The cation-carnitine transporter gene SLC22A16 (solute carrier family 22 member 16) emerged from this screen and its hypermethylation was associated with a favorable prognosis after radio- as well as chemotherapy. Correlation analysis of methylation and expression of SLC22A16 in anaplastic glioma samples and glioblastoma cell lines revealed that the SLC22A16 promoter methylation status is negatively correlated with mRNA expression, indicating a loss of gene function in the hypermethylated form. With SLC22A16 being a L-carnitine transporter, we investigated a possible role of L-carnitine administration on sensitivity towards radiochemotherapy. Interestingly, supplementation with L-carnitine protects human LNT-229 glioma cells from cytotoxic effects induced by radiochemotherapy, rendering them more resistant to glioma therapy. Strikingly, these protective effects are abolished in SLC22A16-knockdown cells, suggesting an important role of SLC22A16 in response to radiochemotherapy. These in vitro findings reflect the data from the NOA-04 trial, with loss of gene function resulting in a better response to glioma therapy. In order to better understand the protective L-carnitine effect we focused on the functional implications of L-carnitine. L-carnitine and its derivatives are thought to foster the cell’s antioxidative potential, e.g. by inducing anti-oxidant genes and reducing the production of reactive oxygen species. Hence, we assumed that cells deficient of SLC22A16 and L-carnitine are more sensitive to oxidative damage induced by radiochemotherapy. In proliferation and clonogenicity experiments L-carnitine showed to have a protective effect on SLC22A16-proficient cells that were challenged with radiochemotherapy, whereas SLC22A16-deficient cells did not seem to benefit from L-carnitine treatment. Subsequently, we further analyzed the connection between L-carnitine and the antioxidative system, focusing on induction of antioxidative enzymes like superoxide dismutase (SOD) and glutathione level, revealing an induction of SOD activity by L-carnitine stimulation. In vivo studies seem to confirm the in vitro data though further analyses in a larger sample size would be useful.