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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 maligant 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-methylatransferase* (*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.