Organotypic slice cultures of human glioblastoma reveal different susceptibility to treatments*

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Introduction

Glioblastoma multiforme (GBM) is an aggressive brain tumor with no efficient treatment at present. We have developed a method to culture human GBM tissue obtained from surgery as organotypic slice cultures which allows for testing the effects of known treatment options including X-irradiation or chemotherapy as well as applying novel compounds as well as Carbon ions [1, 2].

Method

GBM tissue was transported to the lab and processed into organotypic slice cultures as described before . At different timepoints after treatments, slices were fixed, embedded, sectioned and used for morphological evaluation (HE staining, Fig. 2) or analysis of proliferation or cell death with immunofluorescent stainings (Fig. 1).

Results

GBM slices were viable in culture for up to two weeks with preservation of general GBM hallmarks (protein expression, morphologic features) as well as the individual characteristics of the initial tumor. When irradiated with X-rays or Carbon ions, a time-dependent decrease of proliferation in GBM slices was detected (Fig. 1 E and F). This was visualized by using an antibody against Ki-67 which marks cells going through the cell cycle (Fig. 1 A-D).

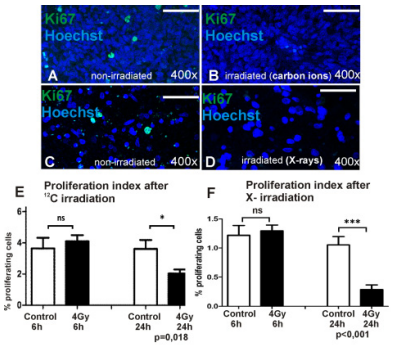

Figure 1: Human GBM slice cultures were irradiated with X-rays or Carbon ions and fixed after 6 or 24 hours. Staining with Ki-67 for proliferating cells revealed a significant decrease in proliferation after 24h. A and C= Control; B= Carbon irradiated (SOBP, 2 Gy); D= X-irradiated (4 Gy); E= proliferation index 6 or 24h after Carbon; F= proliferation index 6 or 24h after X-rays

When treated with the common chemotherapeutic drug temozolomide (TMZ) alone or in combination with irradiation, cell death was induced in slices. Morphological changes and cell loss were visible in HE stainings (Fig. 2 A-D), and apoptosis induction was quantified by staining for activated caspase-3. In the tissues examined here, Carbon irradiation and TMZ induced more cell death and decreased cellular density (Fig. 2 E and F) than treatment with X-rays and TMZ (G and H).

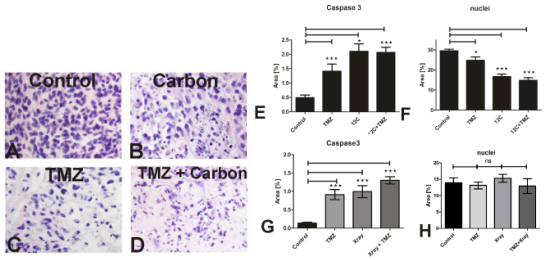

Figure 2: Human GBM slice cultures were treated with TMZ and Carbon ions (SOBP, 2 Gy) or X-rays (4 Gy). In HE stained sections, cell loss and morphological changes are visible (A-D). Fluorescent staining for activated caspase-3 and nuclei revealed induction of apoptosis and cell loss after treatment. The area covered by positive cells in at least 20 pictures per group was analyzed using ImageJ.

The details of this study are now being published in Neuro-Oncology [3].

Discussion

Our model can be used to test effects of different known or novel therapeutic options in one tumor at a time. It can help exposing mechanisms of tumor resistance and cell survival after treatment. In future, it could be used to identify the most suitable therapy strategy for a patient before starting a treatment.

References

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[2] Merz F, Bechmann I. Future Oncol. (2011) 7(4):489-91
[3] Merz F et al, accepted in Neuro-Oncology (2013)

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