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## The Impact of Glioma Invasion and Microenvironment in Development of Radioresistance

Promotionsfach: Radiologie

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An infiltrative growth pattern, necrosis with surrounding pseudopalisades and microvascular hyperplasia are among the key pathological characteristics of glioblastoma multiforme (GBM). Radiotherapy is a cornerstone in multimodal management of GBM patients; however, local recurrence remains the key pattern of therapy failure leading to a dismal prognosis (median survival of ~15 months). This thesis hypothesized that glioma invasion and glioma-stroma communication are causally linked to inherent and acquired radioresistant phenotype of these tumors. Therefore, I first aimed to investigate the radioresponse of glioma cells at non-invasive vs. invasive state. Intriguingly, tumor cells invading the matrigel matrix were found to be less sensitive to irradiation compared to the non-invasive population. Likewise, a relative radioresistance was found in the invasive cell populations generated after sequential *in-vivo* selection of human U87 GBM cells in an orthotopic xenograft model. Increased cell viability, decreased radiation-induced apoptosis and enhanced DNA repair proficiency were attributed to the acquired radioresistant phenotype of invasive vs. matched non-invasive U87 cells. To decipher the molecular mechanisms governing the invasive GBM phenotype a genome-wide *in-vivo* loss of function screen was performed using RNAi technology. Functional knockdown of *LAPTM5*, *LIN7A* and *NUMB* were found to confer glioma invasiveness. Likewise, enhanced tumor invasive fronts were detected after orthotopic implantation of *shLIN7A*- and *shLAPTM5* expressing U87 tumors. On the other hand, overexpression of *LIN7A* reduced *in-vivo* tumor invasion of human glioma initiating cells (GIC) T269. Finally, immunostaining of patient specimens confirmed downregulation of *LIN7A* in glioma cells at the invasive tumor front. Together, these data provide novel insights to the molecular mechanisms governing glioma invasion. The impact of the here identified novel regulators of glioma invasion on development of

radioresistance remain to be elucidated. The candidate glioma invasion-associated genes might constitute novel targets to reverse their radioresistance phenotype. Interestingly, glioma invasion does not render these cells resistance to particle irradiation with carbon ions. Hence, heavy ion irradiation with carbon ions might provide another strategy to circumvent glioma resistance to conventional photon irradiation.

Next, I attempted to evaluate the effects of carbon- vs. photon-irradiation *in-vivo*, with a particular focus on the glioma-stroma interface. Carbon ion radiotherapy is proposed to more efficiently eradicate tumor stem and hypoxic cells compared to conventional photon irradiation, providing a novel strategy in cancer treatment. To further exploit the therapeutic effects of carbon ion radiotherapy, two syngeneic orthotopic glioma models, i.e. murine SMA-560 (*VM/DK*) and GL261 (*C57/BL6*), were employed. The data indicated that current prediction models might underestimate carbon effects at high single doses per fraction. Dose escalation revealed encouraging local controls at  $\geq 15$  Gy carbon dose in SMA-560 model. In contrast to tumor cell centric relative biological effectiveness (RBE) derived from clonogenic survival assay, an enhanced carbon versus proton/photon RBE was found *in-vivo* in SMA-560 model by comparative studies, i.e. iso-doses of 15 Gy photon vs. proton/carbon. In line with this observation, fractionated carbon irradiation ( $3 \times 5$  GyE, equivalent dose based on *in-vitro* RBE) significantly reduced GL261 tumor burden and prolonged survival ( $\sim 2$ -fold) compared to photon irradiation ( $3 \times 5$  Gy,  $p < 0.03$ ). This was in part attributed to an enhanced anti-angiogenic effect (MVD) and reduced recruitment of microglia and Gr1+ myeloid cells correlating with decreased expression of stromal-derived factor-1 (SDF-1) after carbon irradiation. By contrast, inhibiting C-X-C chemokine receptor type 4 (CXCR4) via AMD3100 significantly reduced photon-induced microglia influx in co-culture assay. These results underscored the different modulatory effects of carbon- vs. photon-irradiation on glioma-microenvironment.

In conclusion, the encouraging therapeutic benefits of carbon ions over photon radiotherapy discovered here warrant further preclinical and clinical investigation of this novel therapy modality. Integration of multimodal strategies such as anti-invasion and anti-angiogenesis may further provide rationale to improve the treatment of this invasive, stroma-rich and angiogenic tumor.