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## Brain tumors

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## SUMMARY

In the human brain, a wide variety of primary tumors can be encountered, both in adults and in children. Due to the infiltrative growth pattern of many of these tumors, complete surgical resection is often impossible. Furthermore, eradication of tumor cells by chemotherapy and radiotherapy without damaging the surrounding brain parenchyma is difficult to achieve, and these therapies often result in severe treatment-related side effects. The main obstacles which impair treatment efficacy in these patients are the presence of the blood-brain-barrier (BBB), which prevents drugs to reach clinically-effective concentrations at the tumor location, and the resistance to therapy. In this thesis, we aim to evaluate novel therapeutic strategies to improve the treatment of high grade brain tumors. We explored the use of convection-enhanced delivery (CED) to circumvent the BBB, the use of radiosensitizers to improve radiotherapy and the use of CCR8 inhibition to prevent therapy resistance. Furthermore, we employed a wide range of preclinical imaging techniques to monitor tumor progression.

In **chapter 2** we developed a method to perform CED into the murine brainstem, and to test this method using the chemotherapeutic agent carmustine. We designed a stepped CED-catheter for the delivery of compounds into the brainstem of mice. This CED-catheter was used to treat mice with VUMC-DIPG-3 and E98FM brainstem tumors. Administration of the vehicle solutions (5% dextrose water, or 10% ethanol) into the brainstem was well tolerated. CED of carmustine increased median survival of the tumor bearing mice.

**Chapter 3** describes the use of this CED method for local delivery of the anthracycline doxorubicin to treat pediatric DIPG and thalamic high-grade gliomas. First, the sensitivity of the tumor cells to a panel of anthracyclines was tested *in vitro*. From this panel, doxorubicin was selected for further evaluation. Doxorubicin was formulated either as free doxorubicin (i.e. dissolved directly in the vehicle solution) or in PEGylated liposomes (PEGylated liposomal doxorubicin, PLD). The maximum tolerated dose of these compounds into the thalamus was 10-times higher as compared to delivery into the brainstem. Local delivery of both doxorubicin formulations into the brainstem caused severe toxicity, even at concentrations which were safe when administered into the thalamus.

In **chapter 4**, we show by PET-imaging and distribution studies in orthotopically (intracranial) and heterotopic (subcutaneous) DIPG and high-grade glioma animal models that VEGF expression is dependent on the tumor microenvironment. Tumor cells were injected into different locations, and tumor progression was monitored by bioluminescence imaging and MRI. Although the tumors which were placed subcutaneously showed high accumulation of

$^{89}\text{Zr}$ -bevacizumab, an antibody against VEGF, no accumulation of  $^{89}\text{Zr}$ -bevacizumab was observed in any of the orthotopically located tumors. *In situ* hybridization demonstrated the presence of VEGF in the perinecrotic regions of subcutaneous E98-FM tumors whereas the VEGF-expression in the intracranial tumors was below detection limit.

In **chapter 5**, screening of a library of small-molecule compounds identified the flavonoid quercetin as a radiosensitizer. Radiosensitizing effects were confirmed on medulloblastoma cell lines D283 and D458. Quercetin was shown to sensitize these cells to radiation at low micromolar concentrations. Moreover, quercetin did not affect proliferation of normal human fibroblasts or neural precursor cells. In an orthotopic xenograft model for medulloblastoma, we confirmed that administration of quercetin around the time of radiation increases the radiation efficacy and increased survival of the tumor bearing mice.

**Chapter 6** focuses on the role of extracellular vesicles (EVs). We show that glioblastoma EVs are transferred to recipient tumor cells and can promote tumor progression and resistance to the alkylating agent temozolomide (TMZ). Through RNAi screening, we identified that chemokine receptor CCR8 is involved in EV interaction. We discovered a novel EV-uptake mechanism which involves a triple interaction between the chemokine receptor CCR8 on the cell, glycans exposed on EVs and the soluble ligand CCL18. We demonstrate that EV-induced phenotypes are neutralized by the R243, a small-molecule inhibitor of CCR8. Administration of this compound R243 in combination with TMZ, to mice with orthotopically transplanted glioblastoma, prolongs progression-free survival and prevents TMZ resistance.

In **chapter 7**, we describe the use of optically-cleared, transparent tissues to study the topographical relationship between GBM cells and their microenvironment. We present a workflow for *ex vivo* imaging of optically-cleared brain tumor tissues, and subsequent computational modeling. This workflow was used for quantification of the microvasculature in relation to tumor-cell location in infiltrative GBM models. The detailed 3D-visualization revealed differences of organization of tumor cells relative to the vasculature between grey- and white matter regions. We identified GBM cells co-opting the brain vasculature, cells invading along white matter tracts, and groups of infiltrative, interconnected GBM cells.

In **chapter 8**, we discuss the results of these studies and the relevance of the use of preclinical animal models and imaging techniques in the field of brain tumor research. The insights and techniques described in this thesis could be a start of many new research projects, aiming at the improved treatment of brain tumor patients.