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Intracranial rat glioma model for tumor resection and local treatment

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HIGHLIGHTS

- The intracranial BT4Ca rat glioma model is suitable to simulate tumor recurrence.
- We present a pre-clinical discovery platform for local intratumoral treatment.
- Tumor resection prolonged survival time whereby the tumor regrew in all rats.
- Vehicle application into the resection cavity did not influence tumor recurrence.
- Catheter implantation and intratumoral microinjection did not affect tumor size.

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ABSTRACT

Background: Although tumor resection is among the most important prognostic factors, high grade gliomas regrow in most cases. Also, resection of glial tumors in eloquent brain regions is not or only partially possible. Despite these severe restraints, however, only a few in-vivo models have been established to investigate tumor recurrence and local treatment. Here we characterize the intracranial BT4Ca rat glioma as a model for these aspects.

New method: BT4Ca cells were stereotaxically implanted into the frontal cortex of BDIX rats. Rats were than allocated to (1) a control group, which received no further treatment; (2) a catheter group, where a catheter was implanted for repeated microinjection of vehicle every 3rd day as catheter-control; (3) a resection group, where the tumor was microsurgically removed eight days after cell injection. Postoperatively, survival time, weight and general health condition were scored and the tumor size was histologically assessed.

Results: Injection of BT4Ca cells induced fast-growing tumors with a mean survival time of 16 days in the control and catheter groups. Resection significantly prolonged survival time whereby the tumor regrew in all rats. Tumor size was similar between all groups.

Comparison with existing method(s): We here present a robust and reliable intracranial rat glioma model, which is suitable to simulate tumor recurrence after surgical resection and local treatment. Importantly, this model does not require advanced imaging or elaborate surgical techniques.

Conclusions: The intracranial BT4Ca glioma model appears to be a feasible tool to investigate tumor recurrence after resection and to test local treatment.

1. Introduction

Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults with an annual incidence of more than three per 100000 people worldwide (Zhang et al., 2017). GBM is characterized by an aggressive and invasive growth, resulting in a median survival after diagnosis of less than one year (Johnson and O'Neill, 2012). Standard therapy includes surgical resection followed by radiation and concomitant and adjuvant temozolomide therapy, which, however, increases survival only by few months (Stupp et al., 2005). Despite more recent advances in surgical and concomitant therapy, until now survival time could only be improved to a small extent (Cloughesy et al., 2014; Hottinger et al., 2014; Johnson and O'Neill, 2012; Oertel et al., 2005; Hottinger et al., 2012; Stupp et al., 2009; Weller et al., 2012). Although radical resection still remains one of the most important prognostic factors, tumor

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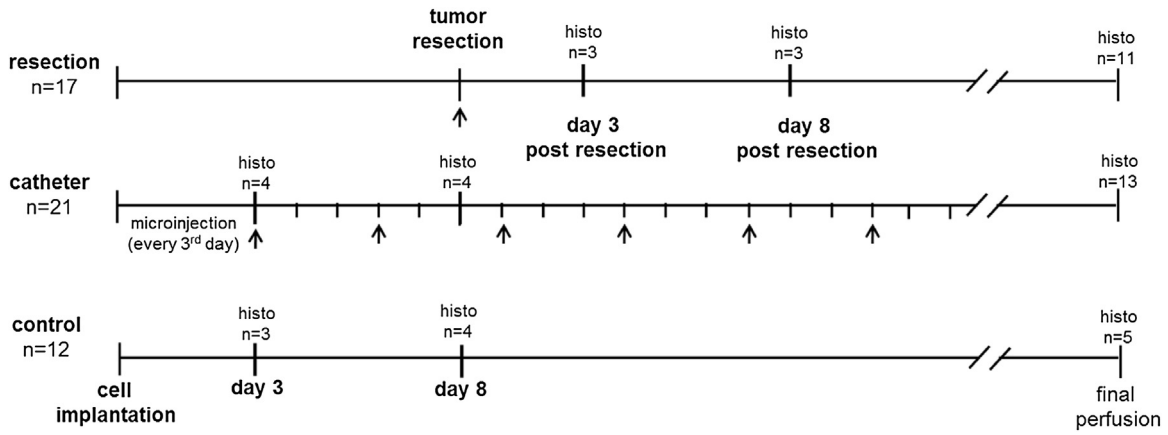

Fig. 1. Experimental design. Timelines show the experimental procedure for the three groups. Time points at which animals were perfused for histological examination of tumor growth are labeled "histo" and the respective number of sacrificed rats is shown. In the catheter group time points for microinjection are indicated by arrows. The arrow in the resection group marks the depot application into the resection cavity.

removal often is not or only partially possible due to the anatomical location, and tumor recurrence is almost inevitable (Butowski et al., 2007; Lacroix et al., 2001).

Because of the blood-brain barrier high doses of drugs are needed with systemic application for effective concentration in the tumor, which is limited by systemic toxicity. Further development of surgical strategies together with novel local treatment methods that overcome the blood-brain barrier therefore has received increased attention (Woodworth et al., 2014). One available local strategy is the implantation of a biodegradable wafer, GLIADEL®, for direct local delivery of carmustine within the resection cavity (McGirt et al., 2009). However, this procedure alone prolongs median survival only for 2.5, or in combination with radiation therapy and temzolomid treatment for 3–4 months (Ashby et al., 2016), and has no benefit in recurrent GBM (Hart et al., 2011). Another approach for local application is convection enhanced delivery, where a catheter is stereotaxically implanted into the tumor or into the resection cavity for direct intratumoral therapeutic delivery circumventing the blood-brain barrier (Debinski and Tatter, 2009).

Rodent models of intracranial tumors may support the development of novel local therapeutic interventions. Several intracranial glioblastoma models are available, which, however, have limitations with respect to the human context (Lenting et al., 2017). One issue is the reliability of tumor formation or the necessity of imaging, which is technically complex and time-consuming in rodents (Bokacheva et al., 2014; Huszthy et al., 2012). The rat BT4Ca glioma model opens new perspectives in this context. The BT4Ca tumor cell line was established by treating immunocompetent BDIX rats with N-ethyl-nitrosourea and is therefore syngeneic to this inbred rat strain. This tumor has been used for a variety of studies to evaluate new therapeutic modalities (e.g., Barth and Kaur, 2009; Huszthy et al., 2006; Pulkkinen et al., 2008; Räty et al., 2004; Sandström et al., 2008). After injection of BT4Ca cells into the frontal cortex of BDIX rats a tumor with infiltrative growth develops with a mean proliferation index of 77% (Borrmann et al., 2013). This tumor has been shown to be susceptible to systemic and local treatment. Intravenous treatment with an α-emitting radionuclide enhanced mean survival time for 2.4 days (Borrmann et al., 2013). Furthermore, local intratumoral application of doxorubicin and irinotecan-eluting beads decreased tumor volume and increased survival time (Baltes et al., 2010; Brinker and Lewis, 2011; Glage et al., 2012).

We here systematically outline the characteristics of the BT4Ca rat glioma model for its suitability as a tumor recurrence model after gross total resection and for its applicability of local targeted therapy, including repeated intracranial microinjection via an implanted catheter and depot application into the tumor resection cavity.

2. Material and methods

2.1. Animals

Adult male BDIX rats (>250 g, bred in the Central Animal Laboratory at Hannover Medical School) were housed in groups of three to four animals per cage with a 12-h light/dark cycle and free access to rat chow and water. The experiments were carried out in accordance with the EU directive 2010/63 and were approved by the local animal ethic committee (Lower Saxony State Office for Consumer Protection and Food Safety, LAVES). All efforts were made to minimize pain or discomfort of the animals used.

2.2. Study design

Rats (n = 50) were divided into three groups. In all groups the experiments started with the implantation of BT4Ca cells (Fig. 1). (1) Rats of the control group (n = 12) received no further treatment; (2) rats of the catheter group (n = 21) were additionally implanted with a catheter for vehicle microinjection every 3rd day, starting three days after cell implantation; (3) the tumor in rats of the resection group (n = 17) was resected on day 8 after cell implantation and vehicle was applied into the resection cavity. Animals of these three groups were sacrificed at defined time points, leading to the following subgroups: rats of the final perfusion subgroup were allowed to survive until reaching humane endpoint criterion of general health condition (control n = 5, catheter n = 13, resection n = 11). Additionally, subgroups of 3–4 rats were perfused on days 3 and 8 after initial cell implantation (control and catheter groups) or after resection (resection group) for histological determination of the tumor morphology and size.

2.3. BT4Ca cell culture

The BT4Ca glioma cells (12th passage from 09.07.2008; Institute of Cell Biology, Department of Cancer Research, University of Essen Medical School, Germany) were cryopreserved in nitrogen at −196 °C. Three days before cell implantation the BT4Ca cells were thawed and incubated (37 °C, 5% CO_2) in Dulbecco's modified eagle medium (DMEM, Biochrom AG Berlin, Germany) containing 10% heat-inactivated fetal calf serum (Biochrom AG, Berlin, Germany) and 1% penicillin/streptomycin (Invitrogen GmbH, Karlsruhe, Germany). After 72 h, the cells were