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Validation of an imageable surgical resection animal model of Glioblastoma (GBM)☆

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HIGHLIGHTS

- Description of a novel cranial defect repair technique in the pre-clinical setting.
- Rat GBM surgical resection model mimics post resection GBM recurrence in patients.
- Modified cranial window method facilitates optical imaging post resection.
- Isolation of surgical resection cavity to allow for local drug delivery.

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ABSTRACT

Background: Glioblastoma (GBM) is the most common and malignant primary brain tumour having a median survival of just 12–18 months following standard therapy protocols. Local recurrence, post-resection and adjuvant therapy occurs in most cases.
New method: U87MG-luc2-bearing GBM xenografts underwent 4.5 mm craniectomy and tumour resection using microsurgical techniques. The cranial defect was repaired using a novel modified cranial window technique consisting of a circular microscope coverslip held in place with glue.
Results: Immediate post-operative bioluminescence imaging (BLI) revealed a gross total resection rate of 75%. At censor point 4 weeks post-resection, Kaplan–Meier survival analysis revealed 100% survival in the surgical group compared to 0% in the non-surgical cohort ($p = 0.01$). No neurological defects or infections in the surgical group were observed. GBM recurrence was reliably imaged using facile non-invasive optical bioluminescence (BLI) imaging with recurrence observed at week 4.
Comparison with existing method(s): For the first time, we have used a novel cranial defect repair method to extend and improve intracranial surgical resection methods for application in translational GBM rodent disease models. Combining BLI and the cranial window technique described herein facilitates non-invasive serial imaging follow-up.
Conclusion: Within the current context we have developed a robust methodology for establishing a clinically relevant imageable GBM surgical resection model that appropriately mimics GBM recurrence post resection in patients.

1. Introduction

Glioblastoma (GBM) is the most frequent and malignant primary brain tumour which accounts for approximately 12–15% of all intracranial neoplasms (David N. Louis, 2007). The disease is heterogeneous on a genetic, cellular and macroscopic level. Moreover, intra-tumoral hypoxic gradients can drive stem cell distribution and effect epigenetic modifications such as MGMT expression.

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Current standard of care for GBM involves surgical resection followed by adjuvant chemo-radiotherapy. Extent of surgical resection provides the single greatest survival benefit for patients, with subtotal resections up to 78% providing a significant survival advantage (Sanai et al., 2011). It has also been demonstrated that surgery can improve the efficacy of adjuvant therapy (Stummer et al., 2011). The survival benefit conferred by surgery may derive from the concept of cytoreduction; by removing the bulk of the tumour and therefore its constituents implicated in resistance and recurrence, thereby improving the efficacy of adjuvant treatment (Jamal et al., 2012; Mannino and Chalmers, 2011; Ng et al., 2007; Shi et al, 2012; Stummer et al., 2011; Tanaka et al., 2011). Surgery also reduces the compressive biomechanical forces of the tumour, which can influence invasion and proliferation of GBM cells and the pharmacodynamics of drugs used (Boucher et al., 1997; Heldin et al., 2004; Shieh et al, 2012; Shieh et al., 2011; Shieh and Swartz, 2011; Tse et al., 2012). Nevertheless, despite maximal treatment, GBM is characterized by tumour recurrence and resistance. Recurrence is due to cell migration from the tumour bulk into normal surrounding brain parenchyma where it is protected by an intact blood–brain–barrier. Resistance is due to complex intracellular and extracellular mechanisms: Genetic and epigenetic factors, tumour cell heterogeneity including the presence of stem cells, as well as certain characteristics of the tumour microenvironment have all been implicated in resistance to treatment.

Within the pre-clinical setting the majority of studies, which employ GBM, rodent models involve a non-surgical therapeutic regimen, and do not consider the therapeutic advantage of surgery, which may significantly reduce the translational context. Herein, we describe the development of a novel, cost effective, imageable rat intracranial GBM surgical resection model. We describe a detailed protocol based on a novel modified cranial window technique to simultaneously repair the craniectomy and permit serial bioluminescence imaging (BLI) over a prolonged follow-up period.

2. Materials and methods

2.1. Cell culture

U87-MG-luc2 GBM cells were purchased from Caliper Life Science (Perkin–Elmer, Hopkinton, MA, USA) were cultured in Eagle's Minimum Essential Medium (EMEM) (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated foetal bovine serum (FBS), 1% L-glutamine (2 mM), 1% penicillin/streptomycin (50 units/ml), all from Sigma–Aldrich (St. Louis, MO, USA). Cells were sub-cultured when the monolayer reached approximately 80% confluency. 0.25% Trypsin-EDTA solution (Sigma–Aldrich, St. Louis, MO, USA) was used to enzymatically detach cells from the surface of the tissue culture vessel.

2.2. Intracranial (i.c.) implantation of U87MG-luc2 GBM cells

10 Foxn1^{rnu} T-cell-deficient, athymic nude rats (Charles River Laboratories, Germany) were selected as they are suitable for GBM tumour xenograft research (Rubenstein et al., 2000). The rats were weighed and anaesthetized *via* intraperitoneal (i.p.) delivery of anesthetic (ketamine (80 mg/kg)/xylazine (10 mg/kg)). Respiration was closely monitored along with the general condition of the animal. Skin was prepared by removing hair with a depilatory cream. The rats were then fixed in a stereotactic frame and the skin was disinfected with alcohol.

A small right parasagittal skin incision was made followed by a craniectomy with a high speed dental drill at the level of the bregma 3 mm right of the midline. After the dura was punctured, 2 μl of cell suspension containing 1×10^5 U87MG-luc 2 GBM cells were aspirated to a Hamilton syringe. The syringe was loaded into the stereotactic arm and cell suspension slowly injected at a depth of 2.5 mm. The syringe was slowly withdrawn. Any residual cell suspension was removed. The skin was closed in a single layer with 4/0 interrupted simple sutures. Animals were monitored post-operatively and returned to their cages when fully recovered. All animal experiments were licensed by the Department of Health and Children, Dublin, Ireland. Protocols were reviewed by the Royal College of Surgeons in Ireland (RCSI) Research Ethics Committee (REC).

2.3. In vivo bioluminescence imaging (BLI)

In vivo BLI was performed using the IVIS Spectrum (Caliper Life Science, Perkin–Elmer Company, Hopkinton, MA, USA). For each imaging procedure, the rats were pre-anesthetized with isoflurane/O_2 mixture. D-Luciferin [d-(−)-2-(60-hydroxy-20-benzothiazolyl)-thiazone-4-carboxylic acid] (Caliper Life Sciences, USA) was formulated in a 30 mg/ml solution of D-PBS w/o Ca^{2+} and Mg^{2+} (Gibco, Invitrogen, Carlsbad, CA, USA), filtered through a 0.22 μm filter, and protected from light. Animals were imaged in identical fashion each week using peak signal time established by kinetic studies. 300 mg/kg of D-Luciferin solution was i.p. injected to each rat 25 min before imaging during whole study. Living Image® Software version 3.2 (Caliper Life Science, Perkin–Elmer Company, Hopkinton, MA, USA) was used to acquire and analyse bioluminescent images. Fixed regions of interest (ROIs) were used to measure tumour bioluminescent signal in each time point.

2.4. In vivo surgical resection

When tumours were in exponential growth phase as determined by bioluminescence signal, animals were randomized into 2 groups: non-surgical (control) and surgical group. Rats were weighed and i.p. delivery of anaesthetic (ketamine (80 mg/kg)/xylazine (10 mg/kg)). No antibiotics were administered for the experiment. Animal respiration and general condition were closely monitored. The skin was prepared by removing hair with a depilatory cream. The rats were then fixed in a stereotactic frame and the skin was prepared in standard surgical fashion.

The previous skin incision was extended in a curvilinear fashion and tissues reflected back. The temporalis muscle was sharply dissected from the cranium and reflected and the pericranium incised and also reflected. The previous burr hole was identified. Using an operating microscope and high-speed dental drill a 4.5 mm craniectomy was made centred on the previous burr hole. A durotomy was made and the tumour located. Under an operating microscope, a macroscopic surgical plane was developed along the brain/tumour interface by enucleating the tumour, which allowed the walls to collapse in. A microdissector was used to further develop this plane and remove the tumour. Hemostasis was achieved with a hand-held electrocautery pen with a fine needle tip. The surgical cavity was irrigated and filled with sterile saline.

To repair the cranial defect and to permit post-operative BLI, 5 mm sterile circular glass microscope coverslips were placed and fixed with cyanoacrylate glue (see Fig. 1). The skin was closed in a single layer over the cranial window with 4/0 interrupted simple sutures and cleaned with alcohol (see Fig. 1). Animals underwent immediate post-operative BLI and were returned to their cage when fully recovered. Animals were weighed daily and assessed for wound infection and general condition. Neurological function was assessed by a previously described method using a modified rat coma scale (Akbar et al., 2009).