Research Article

Development of induced glioblastoma by implantation of a human xenograft in Yucatan minipig as a large animal model

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HIGHLIGHTS

- We developed the U87 glioblastoma model in Yucatan minipig.
- The period of development was short approximately 28 days.
- Minimum blood level of cyclosporine is important to develop GB in Yucatan.
- Brain similarities of minipig and human make it a good model for preclinical studies.
- Yucatan is an affordable animal model regarding the low cyclosporine and caring cost.

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ABSTRACT

Background: Glioblastoma is the most common and deadliest primary brain tumor for humans. Despite many efforts toward the improvement of therapeutic methods, prognosis is poor and the disease remains incurable with a median survival of 12–14.5 months after an optimal treatment. To develop novel treatment modalities for this fatal disease, new devices must be tested on an ideal animal model before performing clinical trials in humans.

New method: A new model of induced glioblastoma in Yucatan minipigs was developed. Nine immunosuppressed minipigs were implanted with the U87 human glioblastoma cell line in both the left and right hemispheres. Computed tomography (CT) acquisitions were performed once a week to monitor tumor growth.

Results: Among the 9 implanted animals, 8 minipigs showed significant macroscopic tumors on CT acquisitions. Histological examination of the brain after euthanasia confirmed the CT imaging findings with the presence of an undifferentiated glioma.

Comparison with existing method: Yucatan minipig, given its brain size and anatomy (gyrencephalic structure) which are comparable to humans, provides a reliable brain tumor model for preclinical studies of different therapeutic methods: in realistic conditions. Moreover, the short development time, the lower cyclosporine and caring cost and the compatibility with the size of commercialized stereotactic frames make it an affordable and practical animal model, especially in comparison with large breed pigs.

Conclusion: This reproducible glioma model could simulate human anatomical conditions in preclinical studies and facilitate the improvement of novel therapeutic devices, designed at the human scale from the outset.

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1. Introduction

Glioblastoma (GB) is the most common and aggressive primary malignant brain tumor in adults (Dréan et al., 2016; Louis et al., 2016) for which no certain cure is available (Burger et al., 1985;
Ferreira et al., 2016), GB leads to death in most patients because of the highly invasive character and infiltration into brain parenchyma (Mujokoro et al., 2016). It is typically confined to the central nervous system (CNS) and does not metastasize outside (Omuro and DeAngelis, 2013). GB accounts for almost 25% of all primary CNS tumors and 55% of all the gliomas with an annual incidence of 3.2 per 100,000 (Allahdini et al., 2010; Thakkar et al., 2014). GB has the highest number of cases among all malignant tumors with an incidence estimation of 12,120 new cases in 2016 (Ostrom et al., 2016). After the first treatment, the majority of all GB patients experience disease progression (Omuro and DeAngelis, 2013). The prognosis of GB is very poor and long-term survivals are rare.

GB management remains palliative and includes the standard treatments of brain tumors, being surgery, radiotherapy and chemotherapy. Cancer immunotherapy is under investigation as an additional treatment modality (Weiss et al., 2016). Today, post-operative radiotherapy combined with concurrent and adjuvant temozolomide (TMZ) as a systemic chemotherapy is the standard treatment method (Hingorani et al., 2012; Johnson and O’Neill, 2012; Omuro and DeAngelis, 2013; Stupp et al., 2005; Walid, 2008). Unfortunately, after an aggressive total surgical resection, GB still remains non-curative because of the infiltrative property of the tumor (Tate and Aghi, 2009; Zagzag et al., 2000). It progresses diffusely and commonly recurs locally within 2 cm of the original tumor bed (Ashby et al., 2016). The therapeutic potential of radiotherapy alone is limited due to the inherent radio-resistance of GB cells (Shafer et al., 2010). Brachytherapy and stereotactic radiosurgery are used for relapsed GB but tend to be associated with notable toxicity (Barani and Larson, 2015). In addition, the blood brain barrier (BBB) is a major limitation, reducing efficacy of anti-cancer drugs in the treatment of GB patients. The passage of anti-tumor agents through the BBB is poorly and heterogeneously documented in the references (Dréan et al., 2016). Despite all efforts to improve the treatment methods, the overall outcome remains poor. With an optimal treatment, the disease remains incurable with a median survival of approximately 12 and 14.5 months after adjuvant radiation and temozolomide-based chemoradiotherapy, respectively (Hingorani et al., 2012; Yang et al., 2015). Beyond this period, the survival rates 2 and 3 years after diagnosis are 26.5% and 2%, respectively (Thomas et al., 2012; Walid, 2008).

These limitations make it essential to administrate high drug concentrations for treatment, potentially exposing patients to severe toxicity and side effects. Therefore, to circumvent the BBB and other restricting factors, novel therapeutic approaches, such as the injection of therapeutic agents directly into the tumor, are advocated to prolong survival time (Cokgor et al., 2000; Kikuchi et al., 2002; Lidar et al., 2004). For this kind of treatment modality, the main issues are the reflux and the leakage after the injection (Buonerba et al., 2011). Reflux is when the fluid flows towards the outside of the tumor through the injection canal. It causes an injected distribution in the tumor that differs from that which was planned. This phenomenon is mainly due to the tumor density. It normally happens if the injection rate exceeds the diffusion rate within the tumor. The second pitfall is the leakage of therapeutic agents during intracerebral injection, which is usually due to the brain ventricles and the cortical sulci near to the injection location (Acabchuk et al., 2015; Selek et al., 2014).

The rodent brain is lissencephalic, meaning that the outer cerebral cortex is smooth and the brain does not contain the sulci (Howells et al., 2010; Semple et al., 2013). This anatomical property, minimizes leakage and improves local drug delivery during infusion in animal experiments (Sampson et al., 2007). Murine animal models, both xenograft and genetically engineered, are most commonly used for cancer research due to the relatively fast generation time (Chen et al., 2013; McNeill et al., 2015; Oh et al., 2014). However their brain lacks the development of the cortex in comparison to primates or larger animals (Sauleau et al., 2009; Semple et al., 2013). The complexity of the human tumor microenvironment and the difference between the brain anatomy of humans and rodents could explain why the majority of successful cancer therapies administered in small animal models cannot be reproduced with humans. This results in a failure in clinical studies and fails to obtain similar efficacious patients (Buonerba et al., 2011; Chen et al., 2013). Developing a cerebral tumor in a large animal model would be very useful in preclinical studies to assess and investigate intratumoral injections in relatively similar conditions to humans. A spontaneous dog glioma model (brachycephalic breeds) exists, but this tumor is quite rare in dogs and constitutes a non-reproducible model (Chen et al., 2013; Dickinson et al., 2010). Among the different large animal species, pigs are an ideal model in settings requiring human-like brain anatomy, histology and vascularization (Lind et al., 2007; Sauleau et al., 2009; Schook et al., 2015). The lissencephalic structure of the pig brain is more similar to human in terms of development compared to the lissencephalic (smooth) brain of common small laboratory animals (Lind et al., 2007). Furthermore, the use of pigs is less expensive and poses fewer ethical concerns than the use of non-human primates, especially when accurate behavioral measurements are not necessary (White et al., 2011). The major benefit of the porcine model for neuroscience research is its brain size. These advantages cause this model to be obviously superior to rodent for preclinical testing and also make it compatible with high-resolution imaging platform used in clinical trials (Sauleau et al., 2009; Snyder et al., 2006). However, the high body weight of mature pigs of large breeds, which can be as much as 250,300 kg, presents an obvious disadvantage. This restriction sets the upper age limit of research pigs at only the first few months after birth. Therefore, in terms of weight, a reasonable alternative to large breed pigs would be the minipigs. An immunosuppressed strain is essential if human cell lines are inoculated as xenografts to generate the tumors and to avoid the rejection of injected cells (Michel-Monigadon et al., 2010). In this study, a minipig model with a human brain tumor is developed by implantation of human GB cell line (U87) in both left and right hemispheres with different cell concentrations to assess the tumor growth and evaluate the imaging and pathological findings. Cells were implanted bilaterally to increase tumor rate in each animal and consequently to decrease for ethical concerns the number of enrolled animals for subsequent research purposes.

2. Materials and methods

2.1. Animals

Yucatan minipigs were selected for the GB development study. Nine animals between 3–4 months old, both male and female, were purchased from INRA Saint-Gilles, France. They were transferred to the Claude Bourgelat institute in Marcy l’Etoile, France to be kept 7–10 days for acclimatization before surgery. The minipigs were regularly examined by a veterinarian to check for any congenital or infectious diseases before administration of the immunosuppressive treatment. The presence of any disease was a criterion for removing the animal from the study. Minipigs were kept at a temperature of 19°C, with humidity >35% and ventilation at least 10 times/hour. The feed was provided by the breeder, with an intake of 350–400 g/day/animal.

2.2. Ethical considerations

The experimental protocol was reviewed and approved by the VetAgro Sup Ethical Committee (1522_V2) and received official authorization by the French Ministry of Scientific Research.
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