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NEOPLASTIC DISEASE

# Spontaneously Arising Canine Glioma as a Potential Model for Human Glioma

**C. Herranz*, F. Fernández†,‡, R. Martín-Ibáñez*, E. Blasco†, E. Crespo*, C. De la Fuente†, S. Añor†, R. M. Rabanal†, J. M. Canals* and M. Pumarola†,‡**

** Department of Cell Biology, Immunology and Neuroscience and Cell Therapy Programme, Faculty of Medicine, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, † Department of Animal Medicine and Surgery, Veterinary Faculty, Universitat Autònoma de Barcelona, Bellaterra and ‡ Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain*

## Summary

Human gliomas are malignant brain tumours that carry a poor prognosis and are composed of a heterogeneous population of cells. There is a paucity of animal models available for study of these tumours and most have been created by genetic modification. Spontaneously arising canine gliomas may provide a model for the characterization of the human tumours. The present study shows that canine gliomas form a range of immunohistochemical patterns that are similar to those described for human gliomas. The *in-vitro* sphere assay was used to analyze the expansion and differentiation potential of glioma cells taken from the periphery and centre of canine tumours. Samples from the subventricular zone (SVZ) and contralateral parenchyma were used as positive and negative controls, respectively. The expansion potential for all of these samples was low and cells from only three cultures were expanded for six passages. These three cultures were derived from high-grade gliomas and the cells had been cryopreserved. Most of the cells obtained from the centre of the tumours formed spheres and were expanded, in contrast to samples taken from the periphery of the tumours. Spheres were also formed and expanded from two areas of apparently unaffected brain parenchyma. The neurogenic SVZ contralateral samples also contained progenitor proliferating cells, since all of them were expanded for three to five passages. Differentiation analysis showed that all cultured spheres were multipotential and able to differentiate towards both neurons and glial cells. Spontaneously arising canine gliomas might therefore constitute an animal model for further characterization of these tumours.



*Keywords:* animal model; dog; glioma; neurosphere

## Introduction

Glioblastoma is the most common primary human brain tumour and it is among the more malignant and difficult to treat of these cancers (Furnari *et al.*, 2007). Its incidence is 2–3 cases per 100,000 people, and the median survival time in treated patients is 12 months (Das *et al.*, 2011). The infiltrative nature of the tumour is a major determinant of its aggressiveness (Rao, 2003). Many efforts are directed to the study and understanding of this devastating malignancy in order to develop effective treatments that may increase the life span of these patients.

Rodents are frequently used to model human gliomas; however, such models are limited by the inbred nature of many rodent strains, differences in life span, size of the animals and lack of spontaneous

Correspondence to: F. Fernández (e-mail: Francisco.Fernandez.Flores@uab.cat).

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development of the disease (Lim *et al.*, 2012). The canine nervous system is much closer in size to that of man, alleviating some concerns regarding scalability and technical limitations such as cellular migration. In addition, dogs have a high incidence of spontaneously arising intracranial tumours (14.5 cases per 100,000 dogs; Stoica *et al.*, 2009). The magnetic resonance imaging (MRI) features of some canine brain tumours have been reported to be similar to those of man (Rodenas *et al.*, 2011; Young *et al.*, 2011). In addition, although smaller in size, the canine brain is microscopically very similar to the human brain (Candolfi *et al.*, 2007; Schneider *et al.*, 2008; Pang and Argyle, 2009; Chen *et al.*, 2013) and canine gliomas are similar to the human equivalents (Candolfi *et al.*, 2007; Vandevelde *et al.*, 2012; Fernández *et al.*, 2015).

The aim of the present study was to further characterize aspects of spontaneously arising canine glioma in order to determine whether this tumour provides an appropriate animal model for investigations of new therapeutic options for human glioma.

## Materials and Methods

### *Case Material*

Gliomas from five dogs were studied, including two oligodendrogliomas, one anaplastic oligodendroglioma, one mixed glioma and one glioblastoma. Dogs were evaluated by a certified neurologist from the Neurology and Neurosurgery Service of the Veterinary Teaching Hospital of the Universitat Autònoma de Barcelona, Barcelona, Spain, and all had neurological signs consistent with a focal brain lesion. Based on MRI of the brain and cerebrospinal fluid analysis, a presumptive diagnosis of glioma was reached in all cases. Breed, sex, age and neurological signs are summarized in Table 1. A healthy, 1-year-old male Beagle dog was used as control. All owners gave their written consent for necropsy examination. All experimental procedures were approved by the Ethics Committee for Animal and Human Experimentation of the Universitat Autònoma de Barcelona.

### *Sample Collection*

Complete post-mortem examinations were performed immediately after death. Serial transverse sections of the brain were made based on MRI images and samples were taken from the centre and periphery of the tumour, contralateral cerebral tissue and the contralateral subventricular zone (SVZ) (Fig. 1). Each sample was approximately 8 $mm^3$. For cell cultures, tissue samples were immersed in control medium (CM) consisting of neurobasal medium containing 100 U/ml penicillin, 100 μg/ml streptomycin and 0.3 mg/ml glutamine (Life Technologies, Madrid, Spain) and preserved in ice until processed.

### *Histological and Immunohistochemical Evaluation*

Samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin (HE). Classification and grading of the tumours was performed according to the criteria defined by the World Health Organization (WHO) for human central nervous system (CNS) tumours (Louis *et al.*, 2007). The microscopical features used for tumour classification were: growth pattern, type and morphology of neoplastic cells, grade of anisokaryosis, presence of mitosis, mucinous secretion, presence of necrosis, vascular features and relationship between tumour and adjacent nervous tissue (i.e. compression or infiltration). Cell markers used for immunohistochemistry (IHC) are shown in Table 2. Anti-mouse (catalogue number K4007; Dako, Glostrup, Denmark) and anti-rabbit (catalogue number K011, Dako) EnVision™ kits were used to label the primary antibodies. In all cases a 3, 3′ diaminobenzidine-based detection kit was used

**Table 1**
**Clinical data from dogs included in the study**

| *Case number* | *Breed* | *Sex* | *Age (years)* | *Clinical signs* | *Location of the tumour* |
|---|---|---|---|---|---|
| 1 | Boxer | F | 8 | Head pressing, compulsive gait, right circling, depressed mental status, absent proprioceptive reactions in left forelimb and left hindlimb, cervical hyperaesthesia | Right prosencephalon |
| 2 | Boxer | F | 9 | Clusters of generalized tonic–clonic seizures every 2 weeks, depressed mental status, right circling, cervical hyperaesthesia | Right prosencephalon |
| 3 | Boxer | M | 10 | Status epilepticus, generalized tonic–clonic seizures | Prosencephalon |
| 4 | French bulldog | F | 4 | Depressed mental status, right hemiparesis, absent menace response in right eye, absent proprioceptive reactions in right forelimb and right hindlimb | Left prosencephalon |
| 5 | French bulldog | M | 4 | Behavioural changes, generalized tonic–clonic seizures | Prosencephalon |

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