The Veterinary Journal 209 (2016) 125–132

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Presence of neural progenitors in spontaneous canine gliomas: A histopathological and immunohistochemical study of 20 cases

Francisco Fernández [a,b,1,*], Alexandra Deviers [c,d,1], Claire Dally [e], Giovanni Mogicato [c,d], Maxence Delverdier [f,g], Laurent Cauzinille [h], Kirsten Gnirs [i], Sònia Añor [a,j], Cristian de la Fuente [a,j], Dolors Fondevila [a], Martí Pumarola [a,b]

[a] Department of Animal Medicine and Surgery, Veterinary Faculty, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain
[b] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain
[c] Université de Toulouse, INP, ENVT, Unité d'Anatomie – Imagerie – Embryologie, F-31076 Toulouse, France
[d] Institut National de la Santé et de la Recherche Médicale (INSERM), Imagerie Cérébrale et Handicaps Neurologiques UMR 825, CHU Purpan, Toulouse, France
[e] Laboratoire d'Anatomie Pathologique Vétérinaire du Sud-Ouest (LAPVSO), F-31201 Toulouse, France
[f] Université de Toulouse, INP, ENVT, Unité d'Anatomie Pathologique, F-31076 Toulouse, France
[g] INRA, UMR 1225, F-31076 Toulouse, France
[h] CHV Frégis, F-94110 Arcueil, France
[i] Clinique Advetia, F-75012 Paris, France
[j] Neurology-Neurosurgery Service of the Veterinary Teaching Hospital of the Universitat Autònoma de Barcelona (UAB-FHCV), Barcelona, Spain

ARTICLE INFO

Article history:
Accepted 13 October 2015

Keywords:
Glioma
Cancer stem cells
Canine
Histopathology
Immunohistochemistry

ABSTRACT

Gliomas are the most common primary brain tumours in humans and are associated with a poor prognosis. An accurate animal model of human glioma tumorigenesis is needed to test new treatment strategies. Dogs represent a promising model because they develop spontaneous diffusely-infiltrating gliomas. This study investigated whether spontaneous canine gliomas contain cancer stem cells previously identified in all grades of human gliomas.

Twenty spontaneous cases of canine gliomas were graded according to the human WHO classification. The expression of different markers of lineage differentiation was evaluated with immunohistochemistry as follows: nestin and CD133 for neural stem cells, doublecortin for neuronal progenitor cells, Olig2 for glial progenitor cells, glial fibrillary acidic protein, vimentin and S-100 for mature glial cells, and NeuN and βIII-tubulin for mature neurons. Gliomas were characterised as follows: five grade II (oligodendrogliomas); nine grade III (seven anaplastic oligodendrogliomas, one anaplastic astrocytoma, one anaplastic oligoastrocytoma); six grade IV (glioblastomas).

Immunohistochemical evaluation revealed that (1) nestin and CD133 were expressed in all grades of gliomas with a higher proportion of positive cells in high-grade gliomas; (2) the expression of S-100 protein and Olig2 did not differ substantially between astrocytic and oligodendroglial tumours, and (3) all gliomas were negative for mature neuron markers. The results demonstrated the presence of undifferentiated neural progenitors in all grades of spontaneous canine gliomas, confirming the relevance of this animal model for further studies on cancer stem cells.

Introduction

Gliomas are the most common primary brain tumours in adult people, representing 81% of all malignant brain tumours (Ostrom et al., 2014). Diffusely infiltrating gliomas are classified as low-grade (grade II) and high-grade (grades III and IV) according to their degree of malignancy (Louis et al., 2007). They are associated with a poor prognosis largely because of their widespread invasiveness and resistance to multimodal treatments.

Recent studies have indicated that all grades of human gliomas contain putative cancer stem cells (CSCs), a small subpopulation of cells thought to be responsible for initiating and maintaining cancer growth through their ability to self-renew (Reya et al., 2001; Singh et al., 2004; Beier et al., 2007; Rebetz et al., 2008). In agreement with the cancer stem cell hypothesis, gliomas are hierarchically organised: self-sustaining CSCs at the apex have the potential to differentiate into astrocytic, oligodendroglial, and neuronal lineages, and give rise to malignant progenitors, lineage-restricted precursors, and differentiated cells (Singh et al., 2004). It is also suspected that CSCs are

* Corresponding author. Tel.: +34 93 5814253.
E-mail address: francisco.fernandez.flores@uab.cat (F. Fernández).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tvjl.2015.10.039
1090-0233/

responsible for resistance to chemo- and/or radiotherapy leading to local relapse after treatment (Bao et al., 2006; Eyler and Rich, 2008). Immunohistochemically, CSCs show expression of markers such as nestin and prominin-1 (CD133) (Singh et al., 2003; Galli et al., 2004; Dell'Albani, 2008).

Efforts are currently being made to decipher the oncogenic mechanisms of these tumours in order to identify new therapeutic targets and improve response to treatment. Although rodent glioma models have been used in preclinical glioma research for over 30 years (Das et al., 2008), their reliability has increasingly been questioned (Sughrue et al., 2009). In contrast, the dog presents an attractive model because of its close evolutionary relationship with humans, its greater brain size compared to rodent models, and the spontaneous nature of gliomas, which are the second most frequent intracranial tumours in this species, with a prevalence of 32% (Chen et al., 2013).

The CSC hypothesis has been investigated in a wide variety of canine tumours (Wilson et al., 2008; Cocola et al., 2009; Fujii et al., 2009; Penzo et al., 2009; Cogliati et al., 2010; Ferletta et al., 2011; Michishita et al., 2011; Nemoto et al., 2011; Blacking et al., 2012; He et al., 2014). However, only one case of canine glioblastoma containing CSCs has been published (Stoica et al., 2009).

Evaluation of lineage commitment of tumour cells in human infiltrating gliomas has demonstrated that low-grade glioma cells are reminiscent of glial-progenitors, while high-grade glioma cells maintain glial progenitor-like features and additionally exhibit enhanced expression of neural precursors (Rebetz et al., 2008). So far, the lineage commitment and differentiation blockage of tumour cells in spontaneous canine gliomas have not been investigated or compared with those aspects of human infiltrating gliomas.

The aim of the present study was to investigate the expression of markers of glial and neuronal lineage differentiation hierarchy in 20 spontaneous canine infiltrating gliomas and to determine whether canine tumours show a lineage commitment similar to their human counterparts.

Material and methods

Case selection

Twenty canine gliomas were retrospectively selected from the databases (2008–2012) of the Veterinary Neuropathology group of the Universitat Autònoma de Barcelona (UAB, Spain) and the Laboratoire d'Anatomie Pathologique Vétérinaire du Sud-Ouest (LAPVSO, France). All of the samples were obtained during necropsy performed immediately following a presumptive diagnosis of glioma. The diagnosis was made by a Board-certified neuroradiologist (SA, CF, LC, KG) based on clinical criteria, magnetic resonance imaging features, and cerebrospinal fluid analysis. All owners gave their written consent for necropsy and histopathological analysis.

Histology and morphological diagnosis

Representative tissue samples were fixed in 10% formalin, processed into 5 µm paraffin-embedded sections, and stained with haematoxylin and eosin (HE) for microscopic evaluation. Gliomas were evaluated by six experts (FF, AD, CD, MD, DF and MP) in a blind study, according to the criteria defined by the World Health Organisation (WHO) for human tumours of the central nervous system (Louis et al., 2007). This classification has been updated more recently compared with the WHO animal grading system (Koestner et al., 1999), and recent veterinary publications have used the human WHO scheme to characterise canine gliomas (Higgins et al., 2010; Young et al., 2011; York et al., 2012; Bentley et al., 2013).

Morphological diagnosis was a two-step process: (1) identification of tumour phenotype and (2) grading. Diagnosis of oligodendroglial tumours relied on the recognition of neoplastic cells with well-defined membranes, cytoplasm clearing, and round and hyperchromatic nuclei, typically organised in a 'honeycomb' pattern. Astrocytic tumours were identified by elongated neoplastic cells with scant eosinophilic cytoplasm, organised in a loosely structured matrix. In oligoastrocytoma, two neoplastic cell populations with astrocytic and oligodendroglial phenotypes, respectively, were intermingled.

High-grade gliomas were distinguishable from low-grade gliomas by an increased degree of cytonuclear atypia (all high-grade gliomas) and an increased frequency of necrosis and/or endoluminal proliferation of endothelial cells leading to glomeruloid-like vessels (all high-grade gliomas except anaplastic astrocytoma). The mitotic index (i.e. number of mitoses per 10 high-power fields) was calculated for each sample but no threshold value was used in the grading scheme. Typical pseudopalisading of neoplastic cells around necrotic foci was a pathognomic feature of glioblastoma. Additional features observed were the growth pattern (relationship with the surrounding tissue), mucinous secretion, and inflammation.

Immunohistochemistry

The immunohistochemical (IHC) markers we used were characteristic of glial and neuronal lineage differentiation hierarchy: nestin and CD133 as stem cell markers; Olig2 protein and doublecortin (DCx) as glial and neuronal progenitor cell markers, respectively; glial fibrillary acidic protein (GFAP) and vimentin (VIM) as mature astrocyte markers; S-100 protein as a mature oligodendroglial and astrocytic marker; and NeuN protein (NeuN) and βIII-tubulin as mature neuron markers. The Iba1 microglial marker was used for the evaluation of the inflammatory-associated response. The nuclear antigen Ki-67 was used as a marker of cellular proliferative activity.

Sections 5-µm thick were mounted on capillary glass slides, deparaffinised, and rinsed with water. The primary antibodies used are summarised in Table 1. When antigen retrieval was necessary, sections were heated for 20 min in a water-bath or

Table 1
Immunohistochemical markers used for the study of canine gliomas: main features.

	Antibody name	Company	Dilution	Pretreatment
Nestin	Rabbit anti-nestin polyclonal antibody	Abcam ab5968	1:500	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
CD133	Rabbit anti-CD133 polyclonal antibody	Abcam 19898	1:200	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
DCx	Rabbit anti-doublecortin polyclonal antibody	Abcam ab18723	1:1000	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
ßIII	Mouse anti-ßIII tubulin monoclonal antibody	Chemicon MAB1637	1:200	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
NeuN	Mouse anti-neuronal nuclei monoclonal antibody	Chemicon MAB377	1:500	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
Olig2	Rabbit anti-Olig2 polyclonal antibody	Chemicon AB9610	1:100	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
GFAP	Rabbit anti-glial fibrillary acidic protein polyclonal antibody	Dako Z0334	1:500	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
S100	Rabbit anti-S100 polyclonal antibody	Dako Z0311	1:1000	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT
VIM	Mouse anti-vimentin clone V9 monoclonal antibody	Dako M0725	1:200	Without pretreatment
Ki-67	Mouse anti-Ki-67 antigen monoclonal antibody	Dako M7240	1:100	Citrate buffer 10 mM pH 6.0, 4 min PC + 30 min RT
Iba1	Goat anti-Iba1 polyclonal antibody	Abcam ab5076	1:600	Citrate buffer 10 mM pH 6.0, 20 min water bath + 30 min RT

RT, room temperature; PC, pressure cooker.