ECRONICON OPEN ACCESS

EC NEUROLOGY
Research Article

Pet Dogs Glioblastoma Model, Literature Review, Findings and Future Applications

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Received: April 29, 2019; **Published:** June 24, 2019

Abstract

Glioblastomas (GBM) are relatively stable in population incidence [24] but the treatment outcome is still not enough satisfying due to their anatomical, histological and genetic features. Interdisciplinary observations have pointed out that family Pet Dogs glioblastoma show extremely similar clinical, radiological, histological characteristics to the Human's one. Considering also that Pet Dogs share the same Love, environment, water and food makes them realistic patients. In this article we review the similar characteristic of both Humans and Dogs GBM observing that "patients" Pet Dogs could become once more Human's best friends by becoming a valid "patient model" for epidemiological, diagnostic and treatment studies for the above mentioned reasons.

Keywords: *Glioblastomas (GBM); Pet Dogs*

Introduction

Gliomas are among the most diffuse intra cranial neoplasms in Humans. Much has been done for the advance in surgical techniques but not much has been acquired in overall survival. This is due to many reasons. The impossibility to eradicate completely the tumour because of the histological architecture of the Central Nervous System (CNS). The need to respect as much normal nervous tissue as possible in order to avoid further neurological deficits is in contrast with the infiltration of cancer cells in the boundaries of the tumor. Another biological characteristic of glioblastoma that leads to difficulty to resect the totality of the tumour in spite of fluorescence surgical technology [25], is the malignant cell infiltration of boundaries. The study of the genetics of these tumors possibly will aid to overcome these problems, although the glioblastoma with it's very high incidence of pleiomorphism , complicates furthermore the study of its genetics. Last but not least glioblastomas are tumors that are relatively rare in comparison to other organ's cancer.

In the recent past many animal models have been proposed [3,5] and utilized but all of them have an unnatural onset of the tumor and often no treatment is proposed.

On the contrary, pet dogs that live in families are part of them and the owner's request of medical-surgical treatment is exactly the same to the one proposed in Humans. Veterinarians modernly treat Pet Dogs following Human protocols, thus leading neurooncologist to collect clinical experimental data as it has been done in the last century in Humans GBM.

Indeed man's best friend lives close to him sharing the similar emotional, nutritional and environmental exposure. These data will become essential for epidemiological, clinical comparison between the two cohort of patients. Dog's tumoral natural history and lifetime is much shorter than Humans, so clinical and eventually experimental therapeutical information can be quickly obtained.

The observation that Humans and Family Dog brain tumours share environmental and life stile per [2,11,13], have similar imaging, genetics and natural history confirms that canine spontaneous glioblastoma is an interesting and realistic model to study [8,14, 10,15,17,19,21]. The object of this paper is to confirm the clinical, diagnostic, genetic features of GBM in order to propose the "Pet Dog MODEL" as a new model for clinical and genetic studies.

Materials and Methods

Ethical consideration and legal considerations

The Dogs analyzed, as reported and mentioned above, are true patients and according to ethical guidelines and legal laws of Italy are submitted to diagnostic and treatment procedures, approved by the government, after that the family signs an informed consent. Since there are no experimental procedures there is no need of ethical committee approval.

The same is for Humans, all patient's are "true patients" and have undergone to standard diagnostic and therapeutic procedures after signing an informed consent.

For the Human patients also an informed consent has been signed for the use of images for this paper.

This paper before submission has been sent for approval to the Veterinary Clinic's and Human Hospital ethical committee. The ethical committee authorized the paper since there are no experimental procedures and for the need of literature review and new ideas in the treatment of Glioblastoma since it is one of the most deadly cancers, even if rare.

In the last 12 months, 5 dogs: 3 boxers and 2 half-breed dogs have been admitted to the veterinary hospital (Ospedale Veterinario San Michele ,Lodi, Italy) as patients on request of the owners, in consequence of epileptic seizure, and after the sign of an informed consent, had undergone to MRI studies without and with contrast enhancement media and so the diagnosis of intrinsic brain tumors was obtained.

The neurological examination was normal.

Image guided stereotactic biopsies confirmed the result of malignant brain tumour (glioblastoma). We emphasize that all dogs in this report are in all aspects patients, with the same rights and expectancy of holistic treatment as Human patients are having.

Two dogs findings were selected for comparison with Human patients findings that were admitted to the Istituto Neurologico C. Besta, for diagnosis and treatments of GBM. At admission all the patients signed an informed consent

The dogs and Humans shared analogue anatomical site of the tumors, MRI images.

Histological Results

The dogs aged 5 and 7 years, the younger is a half breed the elder is boxer. The Human patients are of the same proportional age (52 and 67 yrs) with the same anatomical localization of the lesion. The Dogs and Human Case one had an onset of the disease with epileptic seizure [16], the Human case two, is a recurrent GBM. The Dogs and the Human patients had no antiepileptic therapy before the epileptic seizure. The compared MRI studies are referred to the initial MRI on onset of symptoms. The MRI sequences were T1 weighted sequence, T2 weighted sequence and FLAIR weighted sequence. The veterinary MRI is a Philips 0'5 Tesla meanwhile the Human MRI is a machine for general clinic routine of Philips 1.5 Tesla.

Canine patients underwent a stereotactic image guided bioptic procedure to confirm MRI diagnosis of glioblastoma meanwhile the Human patients underwent to surgical exeresis of the tumor allowing neuropathological studies (We remark once more that all the studies have been performed after the sign of a informed consent and ethical committee approval).

All dogs and Humans had a complete tissue histological examination. Once the tissues were obtained, by biopsy or by exeresis, were immersed in 10% buffered formal saline before routine embedding in paraffin and subsequent microscopic evaluation of 5-µm-thick.

GBM specimens were processed by mechanical dissociation immediately after biopsy or exeresis. They were washed and dissociated by mechanical and enzymatic means. Erythrocytes were lysed using NH4Cl. Isolated cells from the GBM were plated at a concentration of 10^5 cell/ml in matrigel coated culture flasks in a proliferation permissive medium with 1:1 mix of DMEM-F12 and neurobasal medium, B27 supplement 2 Mm (Life Technologies), L-Glutamine, bFGF (10^ng/ml) and EGFR (20^ng/ml) both from R&D Systems as described in literature.

Immunocytochemical staining was done on sections from selected tissues of each case with antibodies to glial fibrillary acidic protein (GFAP), Human von Willebrand factor VIII, MIB-1, (SMA) alpha smooth muscle actin, vimentin and cytokeratins as reported in literature [3,5,10,17,19]. The MIB-1 proliferative index obtained from immunostaining is in percentage and is the result by counting the number of positive coloured nuclei in 1 x 10^3 tumour cells observed from 5 to 10 fields (each 0.16 mm^2) examined at high degree of magnification. The best fields to analyse were the areas that had the highest density of positive reactive nuclei. In addition, using the same immunocytochemical procedure, with appropriate positive and negative controls.

The following antibodies were used [10,17]: 1/50 clone JC/70A from Dako Corp (Carpinteria, CA;) mouse monoclonal antibody to Human endothelial cell CD31; mouse monoclonal antibody 1/200 clone G153-694 BD Biosciences (San Diego, CA;) to Human VEGF; mouse monoclonal antibody to Human EGFR 1/200 Calbiochem Corp (San Diego, CA; clone 158). The Human glioblastoma tissue sections were used in parallel, as positive known controls for immunoreactivity to every staining performed on dogs: the cases of dogs GBM demonstrated to be immunoreactive to the same antibodies.

Results

Neuroradiologic Results

The Dogs and Humans MRI studies showed: consistent peri tumoral edema, sharp borders, ring enhancement, heterogenous T2-weighted signal intensity, iso- to hypointense T1-weighted images, necrosis, and cyst formation.

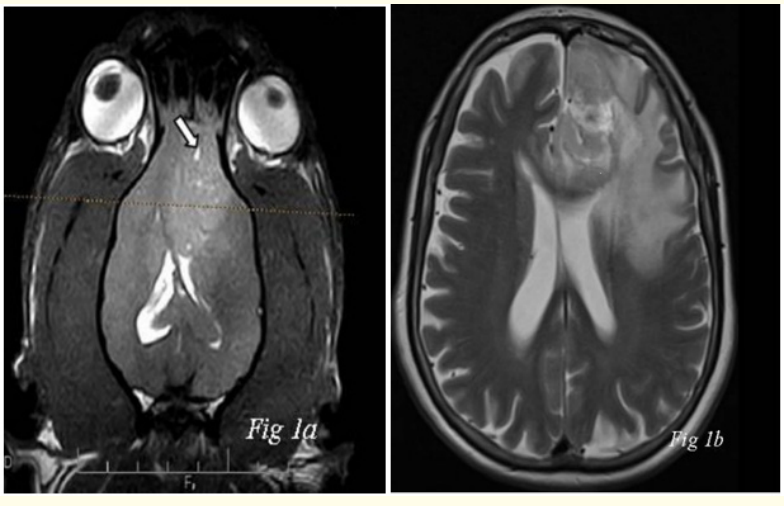

Figure 1: *Brain of dog 1a) Axial T2 weighted MR shows an infiltrative left frontal lobe mass with cystic degeneration (arrow) and significant compression on the ventricular system. Similar situation in the Human brain as shown in figure 1b): Enhanced axial T1-weighted MR shows nodular enhancing with central necrosis.*

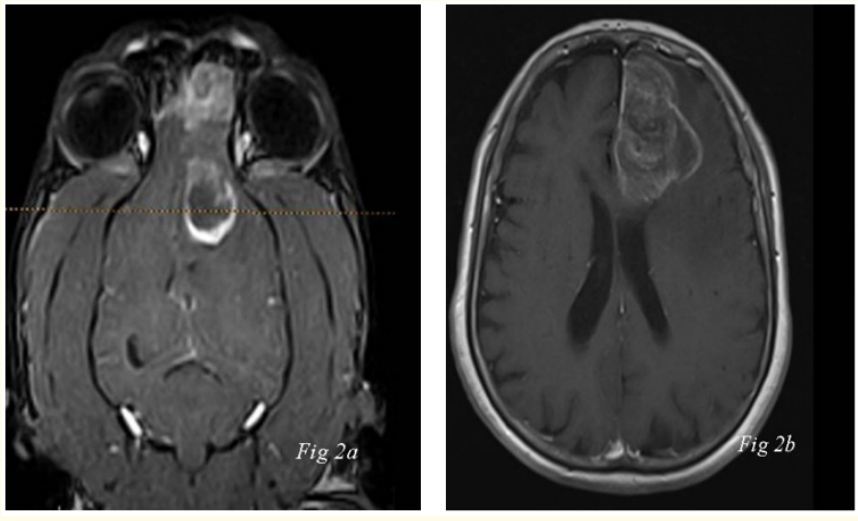

Figure 2: *2a Brain of dog Axial Flair weighted MRI shows a heterogenous mass with extensive abnormal hyperintensity (vasogenic edema) extending into the frontal lobes. Similar situation is present into the Human brain 2b.*

Neuropathological results

The Dog's tissue resembling the Human ones were composed of pleomorphic and anisokaryosis astrocytic tumour cell highly vascularised interlaced with areas of serpentine necrosis surrounded by pseudopalisading glial cells. Larger areas were found with multifocal, microvascular proliferations with acute necrosis. Other areas were irregularly shaped, with thin-walled blood vessels, often with associated haemorrhage. A large amount of tumor cells had typically ovoid, or elongate nuclei with bipolar processes. There were a high mitotic index with both normal and bizarre mitotic figures. The neo angiogenesis included cords of vessels forming multiple tufts and was most represented near areas of necrosis. Immunocytochemical staining of cytoplasmic processes for GFAP and vimentin was strongest in the differentiated astrocytic tumour cells. There were no positive immunoreactivity in any tumours cells for cytokeratins of low and high molecular weight. The MIB-1 immunoreactivity showed a proliferation index of up to 12 - 14% of the tumour cells. Apoptotic cells were concentrated mainly in pseudopalisading cells bordering of necrosis area. Dog and Humans had also a common positive immunoreactivity to EGFR and VEGF [18]. The Dog's staining was of similar intensity and location to the ones in the positive Human GBM control tissue. Positive immunoreactivity for EGFR of tumour cells, but not vascular cells, was observed, it's characteristic was a wide cytoplasmic and membrane staining.

Cancer GBM stem cell

All the primary GBM cultures we generated gave rise to neurospheres within 1-2 weeks called also grow as monolayers upon matrigel coated flasks for more than 35 passages without losing their spherogenic properties. There are already reports in literature of such findings in Humans and in Dogs [17].

Discussion

We have provided a summary of basic diagnostic features that prove that Man's GBM and Dog's GBM are alike in clinical, radiological and histological finding. They are so alike that even the same staining antibodies.

The are Veterinary colleagues experts in neurooncology treat Dog Patients with the same chemotherapy As said before some advances in therapy of glioblastoma multiforme have been done in the last 30 years, but prognosis is still poor. The reasons of this situation are

due to the particular invasion of malignant glioma cells in the CNS and to the lack of a "normal" experimental model. The purpose of the authors was to demonstrate that a "Real Patient Pet Dog Model" is available. Since the Dog's life is naturally shorter that Humans the clinical, histological and radiological features are the same representing a valid realistic model for neuro oncology research.

Conclusion

The findings of this paper highlight a possibility to use the Pet Dog Patient Model in clinical and therapeutic model. But for present and future studies in natural, spontaneous glioblastoma data for many modern therapies could be collected very rapidly. For example, one of the most promising therapies is the immune therapy and many targets [1,6,12,15,17-23] could be studied in Pet Dog Glioma. Obviously, the possibility of experimental treatment, should be discussed with the Dog's family and evaluated by scientific and ethical committees.

What seems to the author that there is a "universal pattern" in cancer that should be used to collect data for a disease that has a such high mortality rate as glioblastoma has. But , at the end of these conclusions, it has to be pointed out that even if there are so many findings that Pet Dogs and Humans share, Humans and Pet Dogs are not the same species and so differences like the immune response and other genetic differences could be a limit to this model.

Disclosure

In the article, the authors must include a draft statement that discloses all relevant conflicts of interest and affiliations. The relevance of financial conflicts of interest with private firms is defined as a relationship of any value with a firm that has a stake in the subject of the manuscript or its competitors.

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Volume 11 Issue 7 July 2019