Chapter

Canine Glioma as a Model for Human Glioblastoma

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Abstract

Glioblastoma, a high-grade diffuse glioma, carries a poor clinical prognosis despite decades of extensive research on the genetic and molecular features of disease and investigation of experimental therapeutics. Because spontaneous canine glioma and human glioblastoma share many clinicopathologic characteristics, recent efforts have focused on utilizing companion dogs as a preclinical model for both research and therapeutic development. A detailed investigation of the canine disease, with particular attention to the genetic and molecular profile, is important in order to allow translation of specific clinical findings from canines to humans and vice versa. In this chapter, we investigate the most common genetic, molecular, and epigenetic alterations associated with canine and human glioma. Appropriate implementation of the canine glioma model may provide valuable information to improve both human and veterinary patient care.

Keywords: glioma, glioblastoma, canine, spontaneous model, translational neuro-oncology, comparative biology, genetics, molecular pathology

1. Introduction

Gliomas are the most common type of malignant primary central nervous system (CNS) neoplasm in humans within the United States [1]. Glioblastoma (GBM), a World Health Organization (WHO) grade IV glioma, is a particularly aggressive tumor, and it accounts for nearly half of the malignant CNS tumors in humans, with an average incidence of over 12,000 cases each year [1, 2]. Even with intensive therapy involving surgery, radiation therapy, chemotherapy, and the most recent FDA-approved therapy utilizing antimitotic alternating electrical fields, the median survival time for patients with GBM is less than 2 years [3].

Companion dogs also spontaneously develop gliomas, including high grade variants that are similar to human glioma and glioblastoma [4]. These canine gliomas share many clinicopathologic features with human disease, such as comparable imaging characteristics, genetic and molecular aberrations, tumor microenvironments, and histopathologic characteristics [5–9]. Correspondingly, the Comparative Oncology Program within the National Cancer Institute developed a Comparative Brain Tumor Consortium (CBTC) to further investigate and utilize spontaneously arising brain tumors in dogs as a model of the human disease with specific emphasis

on comparative glioma [10]. As such, the spontaneous canine glioma model has gained attraction as a preclinical tool to improve the success of human clinical trials by bridging the gap between laboratory models of glioma and human patients.

Subsequent large-scale studies have greatly improved our diagnostic classification and molecular understanding of canine gliomas and have allowed more direct comparisons to human glioma and glioblastoma [11, 12]. While many similarities continue to exist between canine and human glioma, it is also important to characterize the differences between the canine and human disease to ensure that the model is utilized effectively and appropriately. Further investigation into canine glioma, with a focus on comparative molecular and genetic characteristics, can help establish which novel therapeutics can best harness the canine spontaneous glioma model and allow maximal possible benefit to both human and animal patients with gliomas.

2. Overview of canine glioma

Gliomas are the second most common primary intracranial tumor among dogs [4, 13] and have an overall prevalence of 0.9% in the canine population [4]. Gliomas tend to occur in adult dogs, with a median age of diagnosis of approximately 8 years and an increasing prevalence with increasing age [4, 14]. No significant difference in the frequency of intracranial tumors in male versus female dogs have been shown [13], although several recent studies have documented a slightly higher rate of diagnosis in males [14, 15]. Brachycephalic dog breeds, including Boston Terriers, French Bulldogs, English Bulldogs, Boxers, and English Toy Spaniels, are at significantly higher risk of developing gliomas [4] and are overrepresented, collectively comprising 78% of all cases of canine glioma [14]. A recent genome-wide association study identified a genetic locus and 3 candidate genes that are linked to glioma susceptibility in dogs and may have been under selection among brachycephalic breeds [16].

Common clinical signs among dogs with gliomas include: seizure, gait abnormalities, and mentation and behavior changes [14]. Seizures are particularly common among dogs with a specific type of glioma called oligodendroglioma, and these patients are 3 times more likely to experience seizures than dogs with any other type of primary CNS tumor [13]. Cerebrospinal fluid analysis results are variable among dogs with primary brain tumors, as both inflammatory profiles and normal protein and cell counts have been documented in canine gliomas [13, 14]. Although extracranial metastasis of primary gliomas has not been reported in thoracic and abdominal imaging nor post-mortem analysis at necropsy [13, 14], other unrelated concurrent neoplastic processes have been identified both antemortem and at necropsy in canine glioma patients [13].

Computed tomography (CT) and magnetic resonance imaging (MRI) are the two most widely used imaging modalities to aid in the diagnosis and assessment of canine gliomas. MRI is generally considered the preferred modality for identification of intracranial disease, although CT has been shown to detect mass lesions within the brain in 90% of primary brain tumor cases [13] and has similar ability to measure tumor margins as MRI [17]. On MRI, canine gliomas are generally hypointense on T1-weighted images (T1WI) and hyperintense on T2-weighted images (T2WI) [7]; however some reports note that canine gliomas on T1WI and T2WI are also commonly isointense and of mixed intensity [18]. Generally, low grade canine gliomas have lower levels of contrast enhancement, are less commonly associated with cystic structures, and are located more superficially than high grade gliomas [19]. Overall, MRI

is relatively sensitive (approximately 90%) at identifying canine intracranial tumors [18]; however, both MRI and CT are inaccurate predictors of canine glioma type and grade, and ultimately biopsy with histopathology is required for diagnosis [17].

The histopathologic classification scheme of gliomas in both humans and dogs has undergone significant changes in the past several years [2, 11], but generally, gliomas are defined as tumors that resemble glial cells histologically [20]. The two most common types of gliomas are oligodendrogliomas and astrocytomas, and in humans, these gliomas are graded from a scale of grade I to IV based on increasing characteristics of malignancy, as defined by WHO [20, 21]. Molecular and genetic characteristics have been incorporated into the human glioma grading scheme and are expected to be added to the recently revised canine histopathologic glioma classification system [2, 11]. Currently, the three types of gliomas in dogs are oligodendroglioma, astrocytoma, and undefined glioma. These subtypes are then classified as either low- or high-grade based on factors such as necrosis, microvascular proliferation, amount of mitotic activity, and cellular features of malignancy [11].

One important difference between the human and canine disease is the relative frequency of glioma subtypes among patients. The vast majority of human gliomas (approximately 78%) are astrocytic tumors, with 58% of those being the highly malignant GBM [1]. A recent necropsy report utilizing the updated canine glioma classification system reports that astrocytomas may make up as low as 19% of all canine gliomas, with the majority of astrocytomas and oligodendrogliomas being high grade (94% and 84%, respectively) [14]. However, the percentage of canine glioma samples diagnosed as astrocytoma is variable within the literature, with some necropsy reports noting that 35% and 60% of all canine gliomas are astrocytic tumors [4, 13].

Similar treatments options exist for canine glioma, including surgery, radiation therapy, and chemotherapy [15, 22, 23]. In a systematic review of treatment modalities in canine brain tumors, the median survival time of dogs with suspected intracranial gliomas is reported as 226 days [24]. However, euthanasia is also commonly elected for companion dogs diagnosed with gliomas, and one study found that nearly half of all dogs with glioma were euthanized on the day of diagnosis [14]. As such, canine spontaneous glioma is a disease that is associated with significant morbidity and mortality, and novel treatments to improve survival times are clearly still needed.

3. Comparative genetic and molecular signature

Our understanding of the molecular aberrations associated with gliomas has dramatically expanded over the last several decades. In humans, it was found that specific genetic and molecular characteristics are closely linked to glioma biologic behavior and prognosis [20]. Thus, the WHO CNS tumor classification criteria began to incorporate molecular parameters in addition to classic histopathological characteristics into the glioma grading scheme in the 2016 update [2]. In alignment with the goal to utilize canine glioma patients as a model of the human disease, the CBTC assembled a Glioma Pathology Board to revise the canine glioma classification system in a way such that genomic data can be incorporated, mirroring the human classification system [11].

In order to assess the extent to which the spontaneous canine glioma model can be utilized as a model of the human disease, a detailed investigation of what is known about the genetic landscape of canine gliomas is warranted. Genetic alterations that are commonly encountered in human glioblastoma and canine glioma will be discussed, including dysregulation of the receptor tyrosine kinase (RTK)/Ras/

phosphoinositide 3-kinase (PI3K) pathway, the p53 pathway, and the retinoblastoma (Rb) pathway, as well as other specific genes, proteins, and epigenetic factors involved in canine and human glioma. See **Table 1** for a list of abbreviations used for oncogenes and tumor suppressor genes discussed. See **Table 2** for a summary of the comparative somatic mutation rates among common glioma drivers in humans and dogs.

3.1 RTK/Ras/PI3K pathway

Tyrosine kinase receptors are commonly altered in human glioblastoma. Brennan et al. found that at least one RTK is either amplified or mutated in 67% of

EGFR	Epidermal growth factor receptor
PDGFRA	Platelet-derived growth factor receptor A
VEGF	Vascular endothelial growth factor receptor
FGFR	Fibroblast growth factor receptor
NF1	Neurofibromin 1
PTEN	Phosphatase and tensin homolog
PIK3CA	Phosphatidylinositol 3-kinase catalytic subunit alpha
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit 1
TP53	Tumor protein 53
CDKN2A	Cyclin-dependent kinase inhibitor 2A
MDM2	Mouse double minute 2 homolog
RB1	Retinoblastoma 1
CDK4	Cyclin-dependent kinase 4
IDH1	Isocitrate dehydrogenase 1
ATF5	Activating transcription factor 5

Table 1.
Abbreviations of oncogenes and tumor suppressor genes discussed.

	Canine Glioma (Amin et al.) [12]	**Adult Glioblastoma (Brennan et al.) [25]**
EGFR	4%	26%
PDGFRA	21%	4%
NF1	7%	11%
PTEN	<1%	31%
PIK3CA	14%	11%
PIK3R1	1%	11%
TP53	5%	29%
RB1	1%	9%

Table 2.
Somatic mutation rates of selected genes commonly altered in canine glioma and human glioblastoma.

human GBM cases [25]. The most frequently mutated RTK in human GBM is *EGFR* (epidermal growth factor receptor) with a somatic mutation rate of 26%, followed by *PDGFRA* (platelet-derived growth factor receptor A) with a somatic rate of 4% [25]. Both genetic alterations have also been documented in canine glioma; however, the relative frequency is reversed, with a somatic mutation rate of 4% and 21% for *EGFR* and *PDGFRA*, respectively [12]. Utilizing estimates of clonal driver mutations within gliomas, Amin et al. found that clonal *PDGFRA* and *EGFR* mutations occur early on during gliomagenesis within both human and canine gliomas, suggesting molecular similarity among canine and human glioma [12].

EGFR gene amplification is rarely identified in canine glioma, with one report documenting *EGFR* amplification in 3% of cases [8], but overexpression of EGFR protein among dogs with glioma is common. Approximately half of all dogs with gliomas have been reported to have overexpression of EGFR, with significantly greater expression levels among high grade compared to low grade gliomas [26]. Although EGFR mRNA overexpression is seen consistently across both canine astrocytomas and oligodendrogliomas [27], EGFR protein overexpression tends to be more common among astrocytomas and more rarely identified in canine oligodendrogliomas [28].

The *PDGFRA* K385I/M mutation found in a subset of canine gliomas is one of the drivers of glioma in dogs [12]. *PDGFRA* gene amplification is present in nearly half of all canine glioma cases and is particularly common in oligodendrogliomas due to a large gain on canine chromosome 13 [8]. One study found overexpression of PDGFRA mRNA among all canine oligodendrogliomas and nearly half of canine astrocytomas [27]. PDGFRA protein expression patterns are similar, with the highest frequency of PDGFRA overexpression among high grade oligodendrogliomas and fewer numbers of samples overexpressing PDGFRA among canine astrocytomas. Canine astrocytoma PDGFRA overexpression frequency decreases in parallel with decreasing tumor grade [28].

Although genetic alterations in other tyrosine kinase receptors are less common than in EGFR and PDGFRA, many of these receptors have also been investigated as potential targets for glioma therapeutics [29], and will thus be briefly discussed. VEGFR (vascular endothelial growth factor receptor)-1 and VEGFR-2 mRNA overexpression is present in nearly all canine gliomas, with significantly increasing expression correlating with increasing astrocytoma grade [27]. Amplification or mutations involving *FGFR* (fibroblast growth factor receptor) is uncommon in human glioblastoma, with an alteration rate of 3.2% [25], and while the somatic mutation rate for canine gliomas is similarly low, around 1–2% [12], the frequency of *FGFR-1* amplification in canine glioma is notably higher, around 30% [8].

Downstream signaling molecules in the RTK/Ras/PI3K pathway also play important roles in both human and canine glioma and will be investigated further in this section. Somatic mutations involving the tumor suppressor gene *NF1* (neurofibromin 1) occur with similar frequency in human and canine glioma, at a rate of about 11% and 7%, respectively [12, 25]. *NF1* frameshift mutations tend to be late events in the development of gliomas in both humans and dogs [12]. Homozygous losses of *NF1* are uncommon in canine gliomas, being present in about 3% of cases [8]. In a study investigating oligodendrogliomas in brachycephalic breeds, NF1 was not differentially expressed in tumor cells and had similar to expression levels in normal tissue [30].

The tumor suppressor gene *PTEN* (phosphatase and tensin homolog) is the most frequently altered gene in human glioblastoma, with a somatic mutation rate of 31% [25]. Although somatic mutations have not been documented involving *PTEN* in canine gliomas, copy number losses are present in approximately 15% of canine gliomas [8]. With regards to PTEN protein expression, variable expression among

canine gliomas and normal CNS tissue has been observed, with a lack of differential expression in tumor tissue [5, 30].

The second most commonly encountered somatic mutation in canine glioma involves the gene *PIK3CA* (phosphatidylinositol 3-kinase catalytic subunit alpha), which is altered in 14% of cases [12]. *PIK3CA* is also mutated with similar frequency in human glioblastoma, with a somatic mutation rate of 11% [25]. The *PIK3CA* H1047R/L mutation found in a subset of canine gliomas is one of the drivers of glioma in dogs [12]. Mutations involving *PIK3CA* are characterized as early mutations driving tumor formation in canine and pediatric but not adult glioma [12]. However, a closely related gene, *PIK3R1* (phosphatidylinositol 3-kinase regulatory subunit 1), is more frequently altered in human glioblastoma than in canine glioma with somatic mutation rates of 11% and 1% [12, 25].

3.2 p53 and Rb pathways

TP53 (tumor protein 53), a tumor suppressor gene, is one of the most frequently altered genes in human glioblastoma, with a somatic mutation rate of 29% [25]; however *TP53* is infrequently mutated in canine glioma, with a somatic mutation rate of only 5% [12]. Although *TP53* somatic mutations among dogs with glioma are rare, focal somatic copy number alterations are slightly more common, at a rate of 12% [12]. TP53 protein expression is most common in canine astrocytic tumors, with more variable and decreased expression among dogs with oligodendrogliomas [5]. TP53 mRNA expression is upregulated relative to normal tissue in brachycephalic breeds with oligodendrogliomas [30]. *CDKN2A* (cyclin-dependent kinase inhibitor 2A) deletions are commonly seen in human GBM, at a rate of 58% [25]. While *CDKN2A* deletions are also present in canine glioma, these mutations are only in astrocytomas and occur at a lower rate of approximately 12% [12]. Although *MDM2* (mouse double minute 2 homolog) amplifications in canine gliomas have not been documented, *MDM4* is amplified in 42% of canine gliomas [8]. Overall p53 pathway copy number alterations are present in 76% of canine gliomas [8], which is similar to the frequency of p53 pathway alterations in 85% of human glioblastomas [25].

RB1 (retinoblastoma 1) somatic mutations are present in human GBM at a rate of 9% [25], while canine glioma *RB1* somatic mutations are much less common, with only 1% of samples affected [12]. Although *RB1* somatic mutations among dogs with glioma are rare, focal somatic copy number alterations are more common, at an overall rate of 21% [12]. *RB1* deletions are most common among canine oligoastrocytomas, followed by oligodendrogliomas and astrocytomas, with gene losses occurring in 80%, 60%, and 27% of samples, respectively [8]. RB1 protein levels in canine glioma are overexpressed, and most of the RB1 protein is dephosphorylated [5]. *CDK4* (cyclin-dependent kinase 4) is not amplified in canine glioma, and *CDK6* is only amplified in 3% of canine glioma samples [8]. Overall Rb pathway copy number alterations are present in 79% of canine gliomas [8], which exactly mirrors the rate (79%) at which human glioblastomas contain Rb pathway alterations [25].

3.3 Other genetic and epigenetic alterations involved in canine and human Glioma

The classic *IDH1* (isocitrate dehydrogenase 1) R132H mutation commonly seen in human low grade gliomas and secondary recurring human GBM [31] has not been observed in canine glioma [32, 33]. However, mutations involving *IDH1* are found infrequently in canine gliomas, with a mutation rate of 4%, and the *IDH1*

R132C mutation found in a small subset of canine gliomas is one of the drivers of glioma in dogs [12].

The transcription factor ATF5 (activating transcription factor 5), has been shown to be overexpressed in several types of cancers in humans [34, 35], and ATF5 mRNA and protein are overexpressed in human low grade astrocytoma and GBM, with the highest expression in GBMs [36]. ATF5 protein expression is also elevated in canine gliomas, with the highest levels of expression in canine GBM [37].

Canine glioma is reportedly more similar to human pediatric glioma than adult glioma with respect to several different factors. Both canine and human pediatric glioma cases contain at least 1 significantly mutated gene in approximately half of the cases; this is contrasted with adult human gliomas, which carry at least 1 significantly mutated gene over 90% of the time [12]. Although canine glioma has a relatively low mutational burden, aneuploidy (characterized by arm-level copy gains) is common in canine gliomas. The median percent of the canine genome affected by copy number alterations in canine glioma is 25%, which is similar to human pediatric glioma (19–26% of the genome); both of which were higher than adult glioma (8–18% of the genome) [12]. The DNA methylation pattern of canine gliomas was found to be characterized as pediatric glioma in 78% of samples analyzed, with the other remaining 13% and 9% of cases being classified as IDH wild-type adult and IDH-mutant adult glioma, respectively [12].

4. Conclusion

Both human glioblastoma and canine glioma are diseases that carry a grim prognosis for patients. Because dogs develop gliomas spontaneously and with similar frequencies and clinicopathologic features of disease, canine glioma has recently been proposed as a preclinical model for both research efforts and novel therapeutic development prior to clinical trials in humans. In order to best utilize this model, a thorough investigation into what is currently known about canine glioma is of paramount importance.

While many similarities exist between human and canine glioma, several key differences are essential to document so that this model can be used appropriately. The key differences between human and canine glioma that are highlighted in this review include: the relative frequency of glioma histologic subtypes, the frequency of specific genetic variants among drivers of glioma formation, the overall genomic mutational burden, the relative frequency of aneuploidy, and the pattern of DNA methylation. With regards to aneuploidy and epigenetic changes, canine glioma appears to be more similar to pediatric than adult glioma.

These differences are particularly important to consider with respect to investigational therapeutics. New drugs and other therapies that specifically target or otherwise harness these features of glioma to treat the disease may yield different results among canines and humans with gliomas. Additionally, canine glioma may serve as a more reliable model for human pediatric glioma in certain genetic and epigenetic studies.

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Conflict of interest

J.M. Angelastro was on the scientific advisory board member of Sapience Therapeutics (2016–2020), which has licensed the ATF5 technology to treat one of the cancers, glioblastoma, from Columbia University, and is co-inventor on patents owned by Columbia University (New York, NY) and patents owned by Columbia University and the University of California, Davis (Davis, CA).

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