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The genetics of glioma - and the use of dogs as model for human glioma

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Abstract

Glioma is the most common type of primary brain tumor in humans, and the second most common in canines. This tumor type originates from glial cells in the brain and is a genetic disorder caused by mutations in genes regulating important cellular functions. The current diagnosis of glioma is based on histopathological evaluations and gradings. The complexity of the disease requests advanced gene technologies and bioinformatics tools which can aid in the development of new and better diagnosis criteria and therapies. Using Genome wide association studies (GWAS) several genes have been found to be associated with glioma. And with next generation sequencing (NGS) methods, large amounts of genetic information can be produced, stored and analyzed for a low cost. Glioma develops spontaneously in dogs in a similar fashion as in humans and is proposed as a model in glioma research. The findings of new genes associated with glioma can be used for gene, small molecular and immune therapies. Receptor tyrosine kinases VEGFR-1, VEGFR-2, EGFR-1, PDGFR, EGFR and c-MET have been found to be overexpressed in both canine and human gliomas, and growth-factor-targeted therapies have been proposed as treatment for gliomas in canine and humans. Gene therapies including methods as; conditionally cytotoxic therapies, suppression of angiogenesis, immune stimulation, tumor suppressors etc. are progressing in research and clinical trials. No therapy has yet been developed that alone can cure or slow the growth of glioma effectively, but several are in use for complementary treatment in humans. The use of dogs in glioma research and clinical trials can hopefully provide novel findings on how to proceed with more effective therapies and earlier diagnosis. This is a review of the genetics behind glioma and how this information can be used in research for better treatment.

Keywords: c-MET, Canine, Gene therapy, Genome Wide Association Studies (GWAS), Next generation sequencing (NGS), Glioma.

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Abbreviations

APC	Antigen-presenting cell
DC	Dendritic cells
GWAS	Genome-wide association study
HGFR	Hepatocyte growth factor receptor (also called c-MET)
INDEL	Insertion or deletion
LD	Linkage disequilibrium
mAb	Monoclonal antibodies
MHC	Major histocompatibility
NGS	Next generation sequencing
RTK	Receptor tyrosine kinase
SNP	Single Nucleotide Polymorphism
TNF	Tumor necrosis factor
WES	Whole exome sequencing
WGS	Whole genome sequencing
WHO	World health organization
GBM	Glioblastoma multiform

1 Introduction

Gliomas are the most common primary brain tumors in humans and in dogs (Schiffman & Breen, 2015) and are derivied from glial cells in the brain and central nervous system (Goodenberger & Jenkins, 2012). The malignancy of the gliomas is internationally classified according to the World Health Organization (WHO) criteria based on histopathological evaluations and gradings; benign glioma is graded as WHO grade I; Low-grade tumors as grade II; anaplastic as grade III; and the most malignant form of glioma, glioblastoma as grade IV (Louis, 2006).

Despite all the progress that has been made to identify, pathologically characterize and treat gliomas (Ostrom *et al.*, 2014), the survival rate of patients with WHO grade II tumors usually survive 5 years, patients with WHO grade III tumors usually survive 2-3 years and survival of patients diagnosed with the most malignant tumor, WHO grade IV, depend largely upon whether or not treatments are effective and available, but usually not longer than one or one and a half year. The majority of patients diagnosed with glioblastoma do not survive the first year (Louis *et al.*, 2007). There are some concerns regarding the classification scheme based on WHO-grading, the classification is based on visual criteria alone and the categories are not satisfactory for all cases. The classification is subjective and is not necessarily a good predictor of behavior, response or survival as a result of therapies in individual patients and tumors. Thus, an improved classification systems for glioma is desired and may be essential for better outcomes in glioma patients (Louis *et al.*, 2001).

Cancer is caused by genome alterations, for example by several point mutations (*Comprehensive genomic characterization defines human glioblastoma genes and core pathways*, 2008), and the understanding of the genetic basis causing the disease is one of the biggest challenges in biomedical research (Genetic dissection of complex traits, Lander and Schork)(Karlsson & Lindblad-Toh, 2008). Glioblastoma was the first cancer that was studied by The Cancer Genome Atlas (TCGA) (Cancer Genome Atlas Research Network, 2008), and three core pathways were

found to be altered; disrupted growth factor signaling dependent on the receptor tyrosine kinases (RTKs), activation of intracellular signaling of phosphoinositide 3-kinase (P13K) and inactivation of the tumor suppressor genes retinoblastoma (RB) and tumor protein 53 (*TP53*) (*Comprehensive genomic characterization defines human glioblastoma genes and core pathways*, 2008).

The domestic dogs (*Canis lupus familiaris*) share many common diseases with humans including cancer, immune-mediated diseases, cardiovascular diseases and neurological diseases like epilepsy (Hayward *et al.*, 2016). The canine genes that are associated with increased risk of disease development are often orthologous with humans (Karlsson & Lindblad-Toh, 2008). Dogs have been evolved in close relationship with humans often living in the same environment and sharing both space and food sources. In the last two centuries humans have selectively bred dogs with desired morphological or behavioral traits, often resulting in a population bottleneck for different breeds. As a consequence of this artificial breeding and limitations of genetic diversity is somewhat limited, and certain dog breeds have a higher prevalence of specific diseases (Lindblad-Toh *et al.*, 2005). This can be explained by enrichment of risk alleles as a result of random amplification during population bottleneck, or as a result of hitch-hiking mutations near desired traits (Karlsson & Lindblad-Toh, 2008). This suggests that the number of loci underlying the disease is limited and genetic factors associated with disease will be easier to map in dogs than in humans (Lindblad-Toh *et al.*, 2005).

Gliomas occur spontaneously in dogs (Hayes *et al.*, 1975) and share the same histopathological features as human gliomas, which allows similar diagnosis criteria and consequently canine glioma has been proposed as an excellent model for glial cell tumors in human (Schiffman & Breen, 2015). Glioma in dogs is classified using the same WHO grading criteria as in humans. This due to the clinical similarities in the canine glioma compared to human gliomas (Herranz *et al.*). However, canine gliomas are not always classified before treatment is administrated, compared to human gliomas where's a histological diagnosis is required (Bentley *et al.*, 2013). New classification methods is in the same fashion as in human gliomas, essential for better outcome in glioma patients. Studies aiming at defining genes that have abnormal expression in canine glioma have shown an increased expression of several genes encoding receptor tyrosine kinases such as *VEGFR-1*, *VEGFR-2*, *EGFR*, *PDGFR* and *c-MET*, which support the similarities to human gliomas (Dickinson *et al.*, 2006)(Higgins *et al.*, 2009).

In this paper, I will review the findings of gene alterations in canine glioma and how the information can be used for improved diagnosis and therapies in dogs and humans.

2 Literature survey

Cancer is mainly a genetic disorder. Alterations in oncogenes, tumor suppressor genes or DNA repair genes caused by mutations inherited in germ cells or acquired in somatic cells, cause abnormal regulation of cell growth and control mechanism ultimately resulting in tumor development. The uncontrolled growth and cell division increases the risk of additional mutations which can support tumor growth and proliferation (Wong *et al.*, 2011). Hanahan & Weinberg (2011) have proposed eight hallmarks to rationalizing the complexity of cancer. They comprise eight biological mechanisms acquired for development of tumors; sustaining proliferation signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, resisting cell death reprogramming of energy metabolism and evading immune destruction. Each of these hallmarks is a potential therapeutic target. In the last years, other areas have also been proposed as therapeutic targets in cancer including epigenetic alterations and regulatory microRNAs (Hanahan & Weinberg, 2011). Approximately 90% of all known cancer-associated genes are linked to somatic mutations and only 20% are believed to be inherited mutations. Somatic mutations are usually classified as driver and passenger mutations. Driver mutations have an advantage for growth and/or survival, and are positively selected. Passenger mutations have no functional consequence and has not been selected (Wong *et al.*, 2011). Today, malignant gliomas are treated with surgery combined with chemotherapy and radiotherapy. Gaining more knowledge about how certain genes is associated with the development of glioma is important for early detection and for new and more effective personalized therapies (Louis, 2006).

2.1 Genome wide association studies (GWAS)

Genome-wide association studies (GWAS) can be used to scan genomes of case populations and healthy populations as controls to identify regions associated with increased risk for diseas (Sayyab, 2014). GWAS is based on polymorphic markers

that is in linkage disequilibrium (LD) with a specific trait (Truvé, 2012), and uses millions of Single Nucleotide Polymorphism (SNPs) in humans (Edwards *et al.*, 2013) and over 170 000 of SNPs in dogs, who have restricting genetic diversity compared to humans, to map genetic risk factors associated with increased risk for certain diseases. So, if two populations (cases and control) are compared using several markers, and one allele for the case population was found significantly more frequent among the cases compared with the control group, that allele is said to be associated with a trait. However, gene association studies using case and control design have a pitfall, in the presence of subgroups with differing allele frequencies (population stratification) might cause false positive association (Truvé, 2012). Knowledge of genetic variants associated with traits such as certain diseases, can offer markers for early detection, diagnosis and personalized treatment. The majority of identified SNPs (approximately 88%) are located in the intergenic or intronic regions and are most likely to interact with gene regulation (Edwards *et al.*, 2013).

Epigenetics is another important aspect in complex diseases as Glioma. Epigenetics is the regulation of gene expression that is not dependent on the DNA sequence and it can be inherited or acquired (Wong *et al.*, 2011). Epigenetic modifications refer to either DNA methylation of cytosines at CpG dinucleotide or various different histone modifications at particular amino acid residues, including methylation, acetylation and phosphorylation. Both DNA methylation, histone and chromatin modifications have large impact on chromatin structure and gene expression. Epigenetic patterns that increases cell growth and survival are, like driver mutations, positively selected for and is proposed as a target for cancer treatment (Wong *et al.*, 2011). Sequencing studies of glioblastoma, the most malignant grade of glioma, in humans have identified mutations in several genes associated with chromatin modulation including isocitrate dehydrogenase (IDH-1 and IDH-2) that is associated with DNA methylation (Kondo *et al.*, 2014).

2.2 Nucleotide sequencing and Next generation sequencing (NGS)

Sanger sequencing, a first generation sequencing method, have been used for over 40 years in genome research and was the principal method for the completion of sequencing the human genome. The method has also made it possible to identify specific genes that are responsible for development of specific diseases. A newer genome sequencing method is called next generation sequencing (NGS), which can rapidly produce large amount of sequence data and is cheaper than first generation sequencing methods and makes it more obtainable for the average lab (Shyr & Liu, 2013). NGS is based on cyclic array's where the DNA in iterative cycles get treated with enzymatic manipulation to become short reads and is collected as

imaging-based (optical) data every cycle, with multiple sequences read at once. Commercial products that uses this sequencing method includes Roche's 454, Illumina's Genome Analyser, SOLiD and Heliscop from Helicos (Magi *et al.*, 2010). With the giant potential of data that can be obtained from NGS at a relative low cost has made it possible to landscape more complex diseases like cancer. Several collaborative efforts such as the International Genome Consortium (ICGC) (International Cancer Genome Consortium *et al.*, 2010) and The Cancer Genome Atlas (TCGA) projects are currently mapping the genomic landscape in humans. These collaborations will give us a better understanding of the genetics behind diseases, and will take us closer to the goal of developing personalized medicine. Using whole-genome sequencing (WGS) and whole-exome sequencing (WES); point mutations, insertions and deletions (INDELs), repetitions and structural variants can be identified, studied and compared to controls (Meldrum *et al.*, 2011). Illumina HiSeq is one of the most popular choice of platforms for NGS, which uses reversible terminator chemistry (Bentley *et al.*, 2008). However, alternative platforms for next generation sequencing are emerging which are not based on WGS or WES. One example is the Ion Proton platform that is based on semiconductor technologies. This method generates high-quality sequence from large genomes in a relatively short period of time. Bioinformatic tools is then used to collect and handle large amounts of data to understand the complexity of the disease and propose personalized treatment strategies (Sayyab, 2014).

2.3 Bioinformatics – genetic variants

One important challenge in NGS is to align billions of reads accurately and rapid. The massive data collection possible today makes it impossible to use old and traditionally alignment tools. For this reason, several new tools (short read aligners) have been developed that are much faster compared with the traditional aligners. Algorithms are used to quickly and efficiently align the billions of short reads, and most also be able to permit alignment of repetitive sequences, errors and variations. Algorithms today most be improved in order to identify structural variants with higher resolution (Magi *et al.*, 2010). Several different softwares have been published with acquired algorithm criteria (Sayyab, 2014). The search for single nucleotide polymorphism (SNP) and inversion-deletion (INDEL) is important in the research. For example, these programs can also be used to identify loci that are either homozygous or heterozygous, the sequence quality of the obtained data and how well the genomic regions of interest are covered. A conditional likelihood of the nucleotides to exist at a specific position is also predicted using Baye's rules (Magi *et al.*, 2010).

The alignment pipeline is important for a significant result, which includes sorting, merging and improving the alignment by duplicate removal, realignment and base

quality recalibration. Examples of tools used to predict functional significance of SNPs and INDELs are; GATK and SAMtool that can find single nucleotide variants, ANNOVAR software that categorize (annotate) obtained variants into coding or non coding single nucleotide variants and PolyPhen-2 (polymorphism phenotyping) is used to evaluate the functional consequence of missense SNPs. PolyPhen-2 predicts the possible structural and functional effect of single amino acid substitution (Sayyab, 2014).

2.4 Gene therapy and other treatments of glioma

Gene therapy is a collective name for a number of different therapies using genes to treat or prevent diseases.

There are several different suggested treatment options for glioma, including conditionally cytotoxic therapies, suppression of angiogenesis, immune stimulation and correction of mutations in tumor suppressors and oncogenes (Castro *et al.*, 2011). Conditionally cytotoxic therapies introduce a gene encoding an enzyme into the tumor cells, and on the delivery of a noncytotoxic prodrug converts it into a cytotoxic metabolite that will induce apoptosis. One big obstacle is that this enzyme must exclusively be expressed exclusively in tumor cells to be successful (Castro *et al.*, 2011).

Angiogenesis is the growth of blood vessels from pre-existing vessels. The capillaries are important for nutrition and metabolite exchange in all tissues, and essential for growth and proliferation of gliomas. Angiogenesis can be developed by sprouts, also called sprout angiogenesis, on endothelial cells, that grow when stimulated by vascular endothelial growth factor (VEGF-A). Most of these cells can also sense hypoxia and secrete VEGF-A that initiate angiogenesis (Adair & Montani, 2010). During angiogenesis, VEGF-receptors (VEGFR) get activated and induces production of platelet-activation factor (PAF) that increases vascular permeability (Hoeben *et al.*, 2004). One other mechanism of angiogenesis is called intussuspective angiogenesis (also called splitting angiogenesis), this mechanism is less understood compared to sprout angiogenesis, but is a more rapid developed process involving splitting of a single vessel into two. Angiogenesis is important for tumor development, and by inhibition of promoters for angiogenesis such as VEGF the goal is to stop the tumor growth (Castro *et al.*, 2011).

Immune stimulation in cancer therapy is a highly exiting therapy for cancer treatment right now. The immune response that is often elicited in tumors may be caused by tumor-specific antigens. However, most tumors develop a protection against this immune response. There are several cancer therapies aimed at improving the immune response both by gene therapies and as vaccines. One way to stimulate the immune response is by deliver tumor antigens using adenoviral expression. All tumor cells express tumor antigens that are recognized by the im-

mune system. By engineering adenoviral vectors expressing this antigen as vaccines, the hope is to stimulate the immune system. Preclinical trials are showing promising results for some cancers, but have not been tested for gliomas. Another way to stimulate the immune response is to use interferons. Interferons are a group of ligands secreted by the cells in the presence of pathogens and they are involved in inflammation. Due to the highly specific immune response elicited by interferons, they are considered as valuable targets in gene therapy. IFN-α, a type I interferon, has been shown to be an excellent cell cycle inhibitor which also induces apoptosis and stimulate the immune response. IFN-α treatment in human glioblastoma cell lines increases the expression of major histocompatibility complex I (MHC-I) molecules that are involved in antigen presentation leading to activation of the immune system. IFN-α has also been shown to reduce the tumor volumes when overexpressed in mice. There are several other mechanisms for immune stimulation including enhancement of T-cell activities and activation of dendritic cells (DC) (Castro *et al.*, 2011). CD40 is a tumor necrosis factor (TNF) receptor superfamily member, and is expressed on antigen presenting cell (APC) such as B cells, monocytes and DCs. Ligation of CD40 on DCs will for an example increase the expression of MHC, and ligation of CD40 on B cells increase antigen presentation. By using agonistic CD40 monoclonal antibodies (mAb) the goal is to activate APCs followed by anti-tumor specific T cell responses (Vonderheide & Glennie, 2013). The usage of CD40 as an immune stimulator has been tested in spontaneous canine malignant melanoma with good response and further development of clinical treatment is under progress for both dogs and humans (Westberg *et al.*, 2013).

Cancer development, as discussed above, comes from normal precursors but has tendency to mutate key genes, also called driver genes, that regulates proliferation and apoptosis. Tumorigenesis requires mutations in several genes to progress. The genetics in glioma is relatively well established and this knowledge can be used to develop gene therapies to correct these mutations. Some of the pathways with established mutations in human glioma are P53/ARF, receptor tyrosine kinases RTK/RAS and PI3K/PTEN/Akt (Castro *et al.*, 2011). P53 is responsible for regulation of cell cycle and apoptosis. Mutations in this tumor suppressor gene will quickly convert it into an active tumor suppressor and transform cells to become malignant. Every division will increase the risk of new mutations, and with several mutations the risk of cancer development is higher. Due to the fact that cancer is caused by several mutations, correcting mutations in all these genes is most likely impossible and gene therapies aimed at achieving this very complicated. However, in some cases correction of a single mutation can be sufficient to induce apoptosis. One way to correct the mutation is by injecting vectors expressing the P53 wild type protein. Retroviral vectors have been used for gene replacement therapies

with the benefit of only acting on actively dividing cells compared to adenoviruses, for an example, that acts on all cells (Roth *et al.*, 1999).

In canine primary brain tumors, c-MET and other growths factor receptors including receptor tyrosine kinases VEGFR-1, VEGFR-2, EGFR and PDGFR have been reported to be overexpressed in a similar fashion as in humans. This confirms the use of growth-factor specific therapies in dogs and the use of dogs as a model for human glioma (Dickinson *et al.*, 2006).

2.5 c-MET

c-MET (also called hepatocyte growth factor receptor [HGFR]) is one of the most common genes found to be dysregulated in cancer (Liu *et al.*, 2010). *c-MET* is a proto-oncogene that encodes the protein c-MET tyrosine kinase, which is a cell surface receptor expressed in epithelial cells of many organs. The c-MET protein precursor is proteolytically processed in the post-Golgi compartment, where it is cleaved into a 50 kDa α-subunit and a 147 kDa β-subunit that remain attached to each other by a disulphide bond. (Trusolino *et al.*, 2010)(Organ & Tsao, 2011). The extracellular part of c-MET consists of three domains; the N-terminal (500 residues) consists of an α-subunit and parts of the β-subunit and is folded into a semaphorine (Sema) domain, followed by the 50 residue plex-in-semaphorine-integrin (PSI) domain that is connected to four immunoglobulin-plexin-transcription (IPT) domains. The intracellular part of c-MET consists of; the juxtamembrane domain which negatively regulates the enzyme activity by recruiting cCBL using tyrosine Y1003, the kinase catalytic domain positively modulates enzyme activity by tyrosines Y1234 and Y1235, in the c-terminal there is a multifunctional docking site (tyrosines Y1349 and Y1356) which recruit transducers and adapters when c-MET is active. Mesenchymal cells secrete the c-MET ligand hepatocyt growth factor (HGF), also called scatter factor (SF), as a single inactive polypeptide that is activated when cleaved into two (α and β) chains held together by a disulphide bond. HGF act as a multifunctional cytokine and regulates cell growth, proliferation, scattering, survival, motility and morphogenesis by activating the tyrosine kinase signaling cascade (Comoglio *et al.*, 2008). When HGF docks into c-MET it results in homodimerization of the receptor and phosphorylation of tyrosines Y1234 and Y1235, in the subsequent step, tyrosines Y1346 and Y1356, located in the c-terminal of the molecule, become phosphorylated (Ponzetto *et al.*, 1994). The phosphorylated tyrosines Y1346 and Y1356 form a src-homology-2 domain (SH2) that recruit signal-relay molecules that activates several pathways including the growth factor receptor-bound protein (GRB2) – RAS, PI3K – AKT, SRC and RAC1 pathway (Comoglio *et al.*, 2008)(Organ & Tsao, 2011). Another protein that cooperates with c-MET is the hyaluronan recep-

tor CD44 that serve as a linker between the intracellular actin cytoskeleton and the extracellular matrix (Trusolino *et al.*, 2010). HGF and c-MET is especially active during embryonic development, but is also highly active during wound healing and following organ damage for reconstruction of injured tissue (Comoglio *et al.*, 2008). Overexpression of c-MET has been found in several human primary tumors including primary brain tumors (Moriyama *et al.*, 1998). Overexpression of the protein has been reported as a result of heritable activating mutations, but in the majority of humans with increased expression of c-MET, the effect is due to transcriptional up-regulation. HGF that is expressed throughout the body is also able to activate the transcription of c-MET, and support the spread of cancer cells through paracrine positive feedback (Comoglio *et al.*, 2008). In GBM, HGF acting in an autocrine fashion has been shown to activate c-MET transcription (Koochekpour *et al.*, 1997). c-MET's involvement in several pathways and downstream mediators involved in DNA repair and tumor development, makes it a perfect target for cancer therapies.

HGF and c-MET inhibition. Several strategies have been proposed for c-MET inhibition including HGF antagonist and HGF or c-MET neutralizing antibodies. Normally, when the ligand attaches to the c-MET receptor a dimerization causes phosphorylation in catalytic tyrosine residues. This can be prevented by small molecule inhibitors blocking ATP from binding to the kinase active site and prevent phosphorylation and downstream effects (Comoglio *et al.*, 2008). An other inhibitor is a soluble recombinant variant of the extracellular MET domain (decoy MET) that interacts with both c-MET dimerization and HGF interaction with c-MET (Michieli *et al.*, 2004).

Immunotherapy targeting c-MET. Antibodies directed against HGF and c-MET can prevent the binding of ligand to receptor and also induce down regulation of MET. One human monoclonal IgG2 antibody (AMG102) has entered clinical trials phase II against advanced glioblastoma (Buchanan *et al.*, 2011). The results from these trials suggest that AMG102 has a limited effect when used as monotherapy, and later phase trials were not performed. AMG102 will however still be studied in combination trials in human cancers. (Martens *et al.*, 2006)
One problem using antibodies, is their intrinsic agonistic effect and that this can cause activation instead of inhibition of targeted receptor (Martens *et al.*, 2006). Another problem is that resistance can be reached and the tumor become even more persistent (Liu *et al.*, 2010).

2.6 New findings in canine gliomas

Current GWAS, using over 170,000 SNPs, in canine glioma have reported three new genes that are strongly associated with development of glioma. The study was carried out looking at several different breeds. The fact that glioma is overrepresented in certain brachycephalic (short-nosed) dog breeds compared with other breeds, is a strong indication that glioma development is partly caused by genetic factors increased in frequency in the high-risk breeds. This provides an excellent opportunity to identify the genetic risk factors for glioma development by employing GWAS approaches. Two strongly associated SNPs were found in introns of two genes that biologically can be associated with cancer. Gene mapping of canine chromosome 1 (CFA1) and canine chromosome 26 (CFA26), where the two SNPs were found, proposes that one SNP was associated with brachycephaly and the other one with glioma. Across-breed GWAS of the CFA26 locus associated with glioma identified mutations in genes already associated with human cancer (Truvé, 2012).

3 Discussion and future prospects

Dogs and humans diagnosed with malignant glioma have a very low survival rate after five years. Today's treatments using surgery in combination with radiation and chemotherapy is not sufficient and the search for new treatments and classification systems for early diagnosis are highly desired. GWAS and NGS have provided us with important information by identifying genetic risk factors underlying the development of glioma, which opens new possibilities to develop detection and individualized treatment methods at an early stage of tumor development. Several different therapies have been suggested for tumor suppression, and new computational models have been made for prediction of diagnosis, glioma development and prognosis. Gene therapies against glioma constitutes a very exiting prospect, by the delivery of genes into the tumor using viral vectors that will enhance tumor cell death or enhance cytokines such as interferons or tumor necrosis factors that will elicit the immune response against the tumor. Still, gene therapies are in an early stage of development and trials, despite great efforts of researchers all over the world. Gene therapies poses some great challenges; the gene has to be delivered to tumor cells exclusively, the therapeutic gene also needs to be activated in these cells and then avoid getting silenced, the delivery of genes can also trigger the immune system causing serious illness, and genes delivered with the goal of becoming a part of the targeted cell's genome can integrate in the wrong location and disrupt the function of another important gene. The latter example was actually the case in a gene therapy trial of a group of human patients affected by an immunodeficiency. In some of the patients receiving the correcting gene therapy the integrating lantivirus vector integrated within a gene that caused disruption of gene function and subsequent development of T cell lymphomas. This led to a moratorium of this type of gene therapy. Gene therapy in clinical trials is today tested in human patients with already bad survival prognosis, which is a limited number of patients. Good model animals are fundamental for the chance of progressing into further trials.

The domestic dog is an excellent model species for complex human diseases such as glioma, due to their similarities in physiology and natural development of diseases in a similar fashion as in humans. Using dogs as a model would accelerate the genetic studies and drug development due to reduced genetic variations in breeds, shorter lifespan and more offspring's. As a result; mutations, biomarkers and endpoints would be identified more efficiently compared with humans. Using dogs as models would also prevent the risk of harming other model animals by introducing harmful diseases. However, there are some ethical aspects to take into consideration before using dogs as a model, is it morally acceptable to use dogs in research in ways that possibly could cause them harm? There are different ways to look at this; one way where dogs are moral equals to humans and should be treated with the same regulations in research as humans, another way is where there is a moral dividing line between humans and dogs and separate regulations should exist in therapy research. Genetic research minimally requires a clinical examination by a veterinary clinician and that a biological sample, usually a blood sample, is taken from the individual dog, and consequently implicates less physically contacts with the dog patients compared to clinical trials testing new drugs and treatment options, and is therefor often more accepted. All dogs that participate in such studies are privately owned companion dogs and their participation in a genetic study is with the consent of the dog owner. All type of research, genetic or clinical, do at some point comprise a physically procedure such as blood sampling, clinical examination, some times it involves sampling, usually by biopsies or surgery when needed as a clinical procedure to treat the dog from disease. The latter treatment could if problems arise during the surgical procedure, cause the dog harm. Obviously, dogs cannot speak for themselves in the matter, and it is crucial for us humans to take all aspects into consideration and ask ourselves; is the problem worth solving and at what price.

c-MET and other growth factors such as EGFR, PDGFR and IGFBP2, have been found to be overexpressed in both humans and in dogs with glioma. Gene therapies targeting these genes are proposed as treatment for glioma in humans, but have not to my knowledge yet been tested in dogs.
Immune-therapy via activation of the immune response by delivering agonistic CD40 monoclonal antibody (mAb) has been shown to give good response in dogs with malignant melanoma. The fact that CD40 is also expressed in the central nervous system, suggest that this therapy could be a good combined therapy for treatment of glioma.
Further research and trails in dogs would give a better understanding of the effects of gene therapies and prediction of outcomes in humans. Here I propose dogs with

malignant glioma as an excellent model for gene therapies such as growth factor inhibition and Immune stimulated therapies using agonistic CD40 mAb.

To fully use the dog as a model for glioma in humans, cooperation between several experts in both human and veterinary medicine including geneticists, bioinformaticians, pharmacologists, biologists, statisticians etc. is necessary. Because cancer is a complex disease with multiple genome alterations, new high-level analysis methods to find sets of genes that complement each other could speed up the research with the goal of development of improved therapies, better diagnosis and treatments, and ultimately better outcomes for glioma patients, in both humans and in dogs.

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