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IDH1 and IDH2 hotspot mutations are not found in canine glioma

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To the Editor

Human diffuse and anaplastic astrocytomas, well-differentiated and anaplastic oligodendrogliomas, and secondary glioblastomas frequently (>70%) contain somatic mutations of the R132 codon of the cytoplasmic NADP+-dependent isocitrate dehydrogenase (*IDH1*) or the corresponding R172 codon in its homolog, *IDH2*.[1-5] Other gliomas, such as grade IV primary glioblastomas, contain *IDH1* R132 mutations more rarely.[2, 4, 6-8] Less frequent *IDH1* R132 mutations have also been identified in acute myeloid leukemia (AML),[9] prostate cancer,[8] and colorectal cancer.[10] *IDH1* R132 and *IDH2* R172 are conserved in all known species and are important for the enzymatic function of the encoded proteins,[2, 4, 11] but the significance of these changes in cancer is not fully clear.

Canines develop oligodendrogliomas and astrocytomas that resemble the human versions of these tumors. Previously, canine gliomas have been shown to contain common genetic alterations observed in human gliomas, including EGFR expression and amplification, VEGF expression, and aberrant p53 overexpression and gene mutation.[12-14] Of note, IDH1 and IDH2 are highly conserved between dog and human, with 96.9% and 96.4% identity at the protein level, respectively (HomoloGene). The arginines mutated in human gliomas are also conserved between these species, with canine IDH1 R132 corresponding to human IDH1 R132, and canine IDH2 R238 corresponding to human IDH2 R172. The conservation of some genetic mechanisms of gliomagenesis in dogs, the conserved nature of the IDH genes between human and dog, and the high frequency of IDH gene mutations in some human glioma subtypes led us to test whether canine gliomas contain IDH gene mutations.

IDH status was determined for 25 formalin-fixed, paraffin-embedded (FFPE) samples from 25 brain tumors that developed sponaneously. Canine glioma types analyzed included eight oligodendrogliomas, four anaplastic oligodendrogliomas, three astrocytomas, three anaplastic astrocytomas, and two glioblastomas, all of which have been shown to contain IDH gene mutations in humans, as well as one gliomatosis cerebri. Three meningiomas and one anaplastic ependymoma were also analyzed. Genomic DNA was isolated using a

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microtome and the QIAamp DNA FFPE Tissue Kit (Qiagen). H&E slides were created from sections derived from paraffin sections flanking the material used for DNA extraction, and a neuropathologist confirmed the presence of tumor on all slides. Three samples contained 30-50% tumor on both slides, five samples contained 50-70% tumor on both slides, and all other slides contained 70-100% tumor on both slides. For the eight samples with less than 70% tumor tissue identified on either slide, DNA was also isolated from scrapings of the tumor area to analyze pure tumor tissue. PCR and sequencing primers were designed using Primer 3 (http://www-genome.wi.mit.edu/cgibin/primer/primer3_www.cgi) and synthesized by Integrated DNA Technologies (Coralville, IA). Primers were designed to amplify a 202bp region surrounding canine *IDH1* R132, and a 212bp region surrounding of canine *IDH2* R238. PCR amplification and amplimer sequencing were performed using standard methods.

IDH1 R132 and *IDH2* R238 were unambiguously wild-type in all 25 canine tumors analyzed. These results suggest that dog gliomas rarely or never associate with mutations in these codons. We recently showed that canine gliomas contain copy number alterations analogous to those observed in human gliomas. However, the frequency of such alterations often varies between tumors from either species, and analogues for some alterations that are frequent in human gliomas, such as 1p/19q loss in oligodendrogliomas, are not frequent in the canine tumors.[16] Together, these results may indicate that canine tumors develop using mechanisms that are distinct from human tumors with IDH mutations or 1p/19q loss. Alternatively, other unknown genetic alterations may produce the same effect as IDH mutation, 1p/19q loss, or other changes in dogs. Pet dogs that develop spontaneous gliomas have been proposed as a model to test human glioma therapies,[17] and these results emphasize the importance of considering the genetic differences between human and canine tumors for studies using this model.

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