Expression of receptor tyrosine kinases VEGFR-1 (FLT-1), VEGFR-2 (KDR), EGFR-1, PDGFRα and c-Met in canine primary brain tumours

P. J. Dickinson[1], B. N. Roberts[1], R. J. Higgins[2], C. M. Leutenegger[3], A. W. Bollen[4], P. H. Kass[5] and R. A. LeCouteur[1]

[1]Department of Surgical and Radiological Sciences, University of California, Davis, CA, USA
[2]Department of Pathology, Microbiology and Immunology, University of California, Davis, CA, USA
[3]Department of Medicine and Epidemiology, Lucy Whittier Molecular and Diagnostic Core Facility, University of California, Davis, CA, USA
[4]Department of Pathology, School of Medicine, University of California, San Francisco, CA, USA
[5]Department of Population Health and Reproduction (PHK), School of Veterinary Medicine, University of California, Davis, CA, USA

Abstract

Inhibition of tumour growth and angiogenesis by targeting key growth factor receptors is a promising therapeutic strategy for central nervous system tumours. Characterization of these growth factor receptors in canine primary brain tumours has not been done. Using quantitative real-time TaqMan polymerase chain reaction (PCR), we evaluated the expression of messenger RNA (mRNA) for five tyrosine kinase growth factor receptors (vascular endothelial growth factor receptor [VEGFR]-1, VEGFR-2, endothelial growth factor receptor [EGFR]-1, platelet-derived growth factor receptor α [PDGFRα], and c-Met) relative to normal cerebral cortex in 66 spontaneous canine primary brain tumours. Increased expression of VEGFR-1 and VEGFR-2 mRNA was greatest in grade IV astrocytomas (glioblastoma multiforme) and grade III (anaplastic) oligodendrogliomas. EGFR-1 mRNA expression was more consistently increased than the other receptors in all tumour types, while increased PDGFRα mRNA expression was mostly restricted to oligodendrogliomas. The similarities in increased expression of these tyrosine kinase growth factor receptors in these canine tumours, as compared to data from their human counterparts, suggest that common molecular mechanisms may be present.

Keywords
canine, primary brain tumour, TaqMan PCR, tyrosine kinase growth factor receptors

Corresponding address:
P. J. Dickinson
Department of Surgical and Radiological Sciences
Tupper Hall
School of Veterinary Medicine
University of California
Davis
CA 95616
USA
e-mail: pjdickinson@ucdavis.edu

Introduction

Molecular characterization of human primary brain tumours has contributed significantly to a better understanding of tumour biology, development of novel targeted therapies, and the ability to predict clinical progression and response to therapy. All cells contain an integrated network of regulatory pathways that allow diverse cellular events to be controlled via external factors such as peptide growth factors. Activation of these pathways, following the binding of specific growth factors to appropriate receptors, is mediated by phosphorylation–dephosphorylation of protein tyrosine kinases present within the intracellular portion of the receptor. Increased expression of peptide growth factors and their associated tyrosine kinase receptors has been implicated in human brain

tumour proliferation, transformation and angiogenesis. Vascular endothelial growth factor (VEGF) and its major receptors VEGF receptor-1 (VEGFR-1/FLT-1) and VEGF receptor-2 (VEGFR-2/KDR) play a major role in the regulation of tumour angiogenesis, vasculogenesis and vascular permeability in human meningiomas and glial tumours[1–5]. Increased expression of other growth factors and/or their associated receptors including epidermal growth factor[6–13], platelet-derived growth factor (PDGF)[11,14–16] and hepatocyte growth factor/scatter factor and its receptor c-Met[17–19] has also been implicated in angiogenesis and tumour progression in human meningiomas and glial tumours.

In domestic animal species, tumours of the central nervous system occur most frequently in dogs[20–22]. Meningiomas and glial cell tumours (astrocytoma, oligodendroglioma and mixed glioma) are the most commonly reported tumour types[20,23,24], with glial tumours being especially prevalent in brachycephalic breeds such as the Boxer and Boston terrier[20–22]. The incidence of primary brain tumours in dogs, approximately 14.5 per 100 000[25] or 1–3% of all primary neoplasia recorded at necropsy[26], is similar to that in humans, and their biological behaviour, clinical imaging and histological characteristics have many similarities to their human tumour counterparts[21,24,27–31]. However, minimal data are available on the expression of growth factors and their receptors in canine brain tumours, other than the immunohistochemical demonstration of epidermal growth factor receptor (EGFR)[30–32] and VEGF[30] expression in astrocytic tumours. In this study, we measured the expression of the major growth factor receptors VEGFR-1 (FLT-1), VEGFR-2 (KDR), EGFR-1, PDGF receptor α (PDGFRα) and c-Met in spontaneous canine meningiomas, astrocytomas and oligodendrogliomas relative to normal canine cerebral cortex, using quantitative real-time TaqMan polymerase chain reaction (PCR) and correlated the expression with histological tumour type and grade.

Materials and methods

Tissue samples

All tumour tissues were obtained from clinical cases presented to the Veterinary Medical Teaching Hospital, University of California, Davis. Tissues were obtained from surgical biopsy/resections, at necropsy, or from archival paraffin-embedded material. All surgical and necropsy samples were snap frozen and stored in liquid nitrogen. Surgical samples were frozen immediately following excision. Necropsy samples were frozen within 30 min of euthanasia. Whenever frozen tissue was stored, adjacent tumour tissue samples were processed for routine paraffin embedding and histology to provide a microscopic diagnosis. Samples from normal cerebral cortex were collected as controls from both necropsy and archival paraffin-embedded material.

Tumour grading

All tumours were classified and graded by board-certified pathologists (R. J. H., A. W. B.) essentially according to the latest amended criteria of the World Health Organization classification of primary tumours of the human nervous system[33]. Specifically, meningiomas were graded as grade I, grade II (atypical) or grade III (malignant)[33,34]. Oligodendrogliomas were graded as either grade II or grade III (anaplastic)[33]. Astrocytomas were graded as grades II, III or IV (glioblastoma multiforme, GBM).

Canine receptor sequences

Canine complementary DNA (cDNA) sequence data for VEGFR-1 (FLT-1) and VEGFR-2 (KDR) were kindly provided by Dr Rolf Jaussi (Institute of Medical Radiobiology of the University of Zurich and the Paul Scherrer Institute, Switzerland). Canine cDNA sequence for c-Met was kindly provided by Dr Cheryl London (The Ohio State University College of Veterinary Medicine). The cDNA encoding canine EGFR-1 sequence spanning exons 15–20 (GenBank accession AY527212) was obtained by PCR from normal canine brain and liver cDNA using primers derived from rat EGFR-1 sequence (GenBank accession NM031507). The cDNA encoding canine PDGFRα sequence spanning exons 11–18 (GenBank accession AY525124) was obtained by PCR from normal canine brain and liver cDNA using primers

derived from human and murine PDGFRα sequence (GenBank accessions D50007 and NM 011058).

RNA extraction/cDNA preparation

Total RNA was extracted from 30 μm sections of formalin-fixed, paraffin-embedded tissue and reverse transcribed into cDNA as previously described[35]. The cDNA was analyzed immediately or stored at −20 °C until use. For nucleic acid extraction from snap-frozen surgical and necropsy tissues, 20–25 mg of samples were transferred frozen into 96 well plates containing two grinding beads (4 mm in diameter; SpexCertiprep, Metuchen, NJ, USA), immediately homogenized (GenoGrinder2000; SpexCertiprep) and total RNA extracted from the tissue lysates using a 6700 automated nucleic acid workstation (Applied Biosystems, Foster City, CA, USA).

Real-time TaqMan PCR

Expression of FLT-1, KDR, EGFR-1, PDGFRα and c-Met messenger RNA (mRNA) in brain tumour samples relative to normal brain was determined using real-time TaqMan PCR.

TaqMan PCR systems for canine housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ribosomal protein L13A, glycosyltransferase (HPRT1), and glucuronidase beta (GUSB), were designed and validated as previously described[36]. Target gene systems for VEGFR-1, VEGFR-2, EGFR-1, PDGFRα and c-Met were designed based on canine sequences described above using Primer Express software (Applied Biosystems) to standardize reaction conditions and cycling requirements (Table 1). The internal probe was labelled at the 5′ end with the reporter dye FAM (6-carboxyfluorescein) and at the 3′ end with the quencher dye TAMRA (6-carboxytetramethylrhodamine). To allow discrimination between cDNA and genomic DNA, either one of the PCR primers or the TaqMan probe was placed over an exon–exon junction. The PCR reactions contained 400 nM of each primer, 80 nM of the TaqMan probe and commercially available PCR mastermix (TaqMan Universal PCR Mastermix; Applied

Table 1. TaqMan PCR primer and probe sequences

Target	Forward primer sequence (5′→3′)	Reverse primer sequence (5′→3′)	Length (bp)	Probe sequence (5′→3′)
GAPDH	GATGGGCGTGAACCATGAG	TCATGAGGCCCTCCACGAT	131	CCCTCAAGATTGTCAGCAATGCCTCCT
Ribosomal protein L13A	AAGTTGAATTACCTGGCCTTCCT	GGGTGCCCGGAAGTCATAG	78	AACACCAACCCATCCCGTGGCC
Glycosyltransferase	GAGATGACCTCTCAACTTTAACTGAAAA	CAAGGGAAGCAAGGTTTGCA	92	CTTGATTGTTGAAGATCTCATTGACACAGGCA
Glucuronidase b	GCTGGATCAGAAACGCAAAGA	CTCTCTGTGGTGACTGGTCAGTCAT	86	TGGTTGGAGAGCTCATCTGGAATTTTGCT
VEGFR 1 (FLT-1)	GCTGTGCGCGCTGCTT	AACTCAGTTCAGGACCTTTTAATTTTGA	77	CGGCCTGCTGCTCACAGGATCTAGTT
VEGFR 2 (KDR)	TGATACTGGAGCCTACAAGTGCTT	CCTGTAATCTTGAACGTAGACATAAATGA	79	ATCGGGACACTGACATGGCCTCG
EGFR	TGCTGCAAGAAAGAGAGCTTGT	AAGATCCTCAAGAGAGCCTGGTT	76	CCTCTTACACCCAGCGGAGAAGCTCC
PDGFRα	CCCACGCTGCGTTCTGA	TCATACCTCGGTTTCTGTTTCCAA	113	TGCAGTCCTGGTGCTATTGGTGATTGTG
c-Met	TGCTGGTGTTATCTCAATATCAACAA	CACTGCCCAGATCTTTAATTTGCTT	95	AGTCTTATTATTACTCGGACTTTTCCTGTGGCTGAA

Biosystems) and 5 μL of the diluted cDNA sample in a final volume of 12 μL. The samples were placed in 96 well plates and amplified in a combined thermocycler/fluorometer (ABI PRISM 7700 SDS; Applied Biosystems). Amplification conditions were 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. Final quantitation was done using the comparative C_T method and was reported as relative transcription or the *n*-fold difference relative to a calibrator (mean value for individual normal cerebral cortex samples). C_T values for GAPDH for each tumour group were averaged and compared groupwise. Individual samples that showed significantly lower C_T values for GAPDH (cut-off 3 C_T values weaker than average) were considered low quality cDNA samples and eliminated from the analysis.

Statistical analysis

Data were divided into nominal categories of tumour type, and within these types, they were divided into ordinal categories of grade. Kruskal–Wallis one way analysis of variance was used to compare the variation in expression of canine receptor tyrosine kinase mRNA among tumour types. When significant differences were evident, Mann–Whitney tests were used for pairwise comparisons. Within tumour type, evidence of a trend in expression across ordinal grades was evaluated using the Jonckheere–Terpstra test. Side-by-side box plots were used to nonparametrically summarize the distribution of the dependent variables. Statistical significance was defined as $P < 0.05$.

Results

A total of 15 samples of normal cerebral cortex (4 frozen and 11 paraffin-embedded) and 66 tumour samples (13 frozen and 53 paraffin-embedded) were analyzed. Of these samples, there were 23 meningiomas (9 grade I, 13 atypical and 1 malignant), 23 astrocytomas (7 grade II, 8 grade III and 8 GBM) and 20 oligodendrogliomas (2 grade II and 18 grade III). For all genes analyzed, the averages of normalized values and the standard deviations for both frozen and paraffin-embedded samples were not significantly different.

VEGFR-1 (FLT-1) expression

Product was amplified from 10 meningiomas, 18 astrocytomas and 20 oligodendrogliomas (Fig. 1A). Increased expression was seen predominantly in glial tumours, with the highest expression in GBMs and oligodendrogliomas. Expression in high-grade (III) oligodendrogliomas was significantly increased relative to low-grade astrocytomas (II) ($P < 0.0002$) and meningiomas ($P < 0.0001$). Expression in GBMs was significantly greater than meningiomas ($P < 0.00072$), and there was a significant trend of increasing expression with increasing grade of astrocytic tumour (grade II, grade III and GBM) ($P < 0.010$).

VEGFR-2 (KDR) expression

Product was amplified from 19 meningiomas, 20 astrocytomas and 20 oligodendrogliomas (Fig. 1B). As with VEGFR-1, increased expression was seen predominantly in glial tumours. Expression in GBMs and oligodendrogliomas was significantly greater than in meningiomas of all grades ($P < 0.045$ and $P < 0.032$, respectively). Expression in high-grade meningiomas (atypical/malignant) was significantly greater than in grade I meningiomas ($P < 0.00024$).

EGFR-1 expression

Product was amplified from 17 meningiomas, 19 astrocytomas and 20 oligodendrogliomas (Fig. 1C). Increased expression of EGFR-1 was found in all tumour types; however, the majority of grade I meningiomas had low expression relative to other tumour types and high-grade meningiomas. Greatest expression was seen most consistently in the high-grade gliomas (GBMs and oligodendrogliomas). Expression in oligodendrogliomas was significantly greater than in grade I meningiomas ($P < 0.0001$).

PDGFRα expression

Product was amplified from 10 meningiomas, 11 astrocytomas and 20 oligodendrogliomas (Fig. 1D). Increased expression was most often found

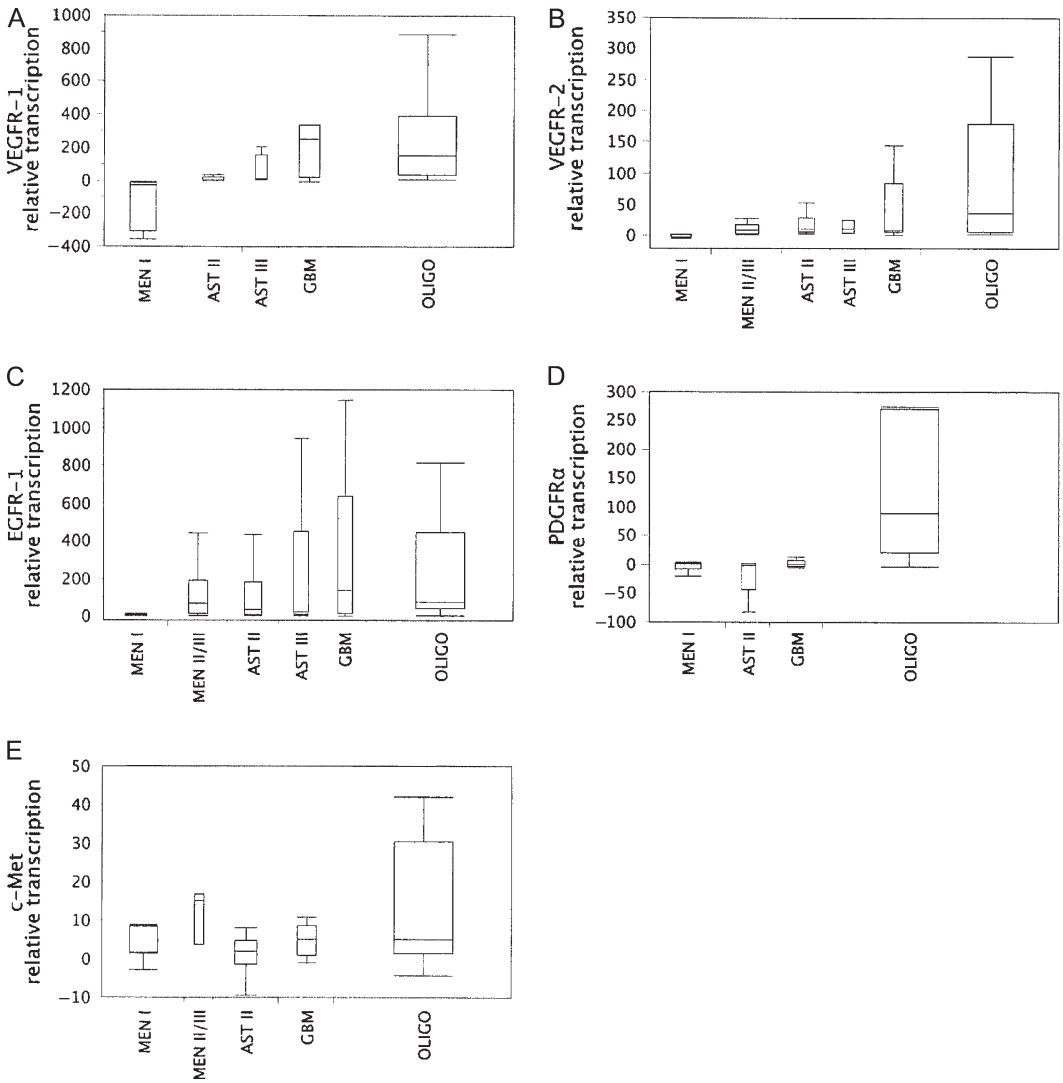

Figure 1. Box plots of growth factor receptor transcription levels. The top and bottom edge of the box represent the upper and lower quartile, respectively. The line within the box represents the median. The tails extend to the farthest point that is within 1.5 interquartile ranges of the quartiles. *x*-Axis divisions are proportional to sample size. (A) VEGFR-1 (FLT-1) expression, (B) VEGFR-2 (KDR) expression, (C) EGFR-1 expression, (D) PDGFRα expression and (E) c-Met expression. MEN I, meningioma grade I; MEN II/III, meningioma grades II/III; AST II, astrocytoma grade II; AST III, astrocytoma grade III; GBM, glioblastoma multiforme OLIGO, oligodendroglioma grade III.

in oligodendrogliomas. Expression in oligodendrogliomas was significantly greater than in GBMs, grade II astrocytomas and grade I meningiomas ($P < 0.0004$, $P = 0.0003$ and $P < 0.0001$, respectively).

c-Met expression

Product was amplified from 12 meningiomas, 13 astrocytomas and 19 oligodendrogliomas (Fig. 1E). Trends of increasing expression with increasing tumour grade occurred in both meningiomas and astrocytic tumours, although not a statistically significant difference. The greatest increases in expression occurred in oligodendrogliomas.

Discussion

Increased expression of both mRNA and protein has been demonstrated previously for VEGFR[3–5], PDGFR[14–16,37], EGFR[8,9,13] and c-Met[17] in human astrocytomas, oligodendrogliomas and

meningiomas. Based on these data, experimental therapies targeting defined growth factors and their tyrosine kinase receptors have progressed into human clinical trials[38]. Effective translation of these therapeutic strategies into canine brain tumours will require identification of similar molecular abnormalities. Little information is available relating to expression of growth factor receptors in spontaneous canine cancer of any type. Increased expression of VEGFR-1 has been described in canine mammary tumours, melanomas, fibrosarcoma, mastocytoma and pancreatic carcinoma[39–41], while reports of increased EGFR and c-Met expression have been limited to canine mammary tumours and osteosarcomas, respectively[42–45]. The data presented suggest that altered tyrosine kinase growth factor pathways including VEGFR-1, VEGFR-2, EGFR, PDGFRα and c-Met may be present in canine meningiomas and glial tumours, similar to those in their human tumour counterparts.

VEGFR expression

Increased expression of VEGFR-2 was found in human meningiomas, astrocytomas and oligodendrogliomas, with greatest expression in high-grade glial tumours[2–5]. A similar pattern of expression has been reported for VEGFR-1; however, the data are less consistent[2,4,5]. The pattern of expression of VEGFR-2 mRNA in canine tumours was very similar to those in their human counterparts, with the greatest expression seen in grade III oligodendrogliomas and GBMs and lower expression in meningiomas. VEGFR-1 expression was also greatest in high-grade gliomas similar to equivalent human tumours. Expression of VEGF receptors is rarely seen in normal brain vasculature and is predominantly located in endothelial cells within tumour vasculature[3,5]. However, expression has also been reported in non-vascular elements of tumour tissue from both human brain tumours[3,46] and canine tumours[39,40]. Expression of VEGF receptors is induced during tumour progression and probably plays a major role in tumour angiogenesis[3]. Histological assessment of microvascular density was not done in this study; however, the greatest expression of VEGF receptors was seen in high-grade gliomas, where microvascular proliferation is a specific criterion for tumour grading. The significance of decreased VEGFR-1 mRNA expression in some canine tumour samples (particularly meningiomas) is unknown; however, it has been suggested that the function of VEGFR-1 may be inhibitory via regulation of VEGF bioavailability following ligand binding to alternatively spliced soluble receptors[47]. Downregulation of VEGFR-1 may provide a biological advantage for tumour growth in this situation.

EGFR expression

In human glial tumours, increased EGFR-1 expression was seen predominantly in high-grade astrocytomas, usually as a result of gene amplification and/or gene rearrangement[6–9], and in oligodendrogliomas[9,10]. Increased expression was seen in meningiomas[9,13], although at a lower level[9], with no consistent association between increased EGFR-1 expression and meningioma grade or histological subtype[11,12].

In this study, increased EGFR-1 expression was also present in all canine tumour types and grades and was a more consistent finding than for the other receptors (VEGFR, PDGFRα and c-Met) where increased expression was most typically seen in high-grade gliomas. Increased EGFR-1 expression has been reported in a small number of low-grade human astrocytomas[8,9], and this was also found in canine low-grade astrocytomas. Similar findings were reported for EGFR-1 by Stoica *et al.*[31], where three of six canine astrocytomas with positive immunostaining for EGFR-1 were low-grade (II/III) astrocytomas.

PDGFR expression

Increased expression of PDGFRα, PDGFRβ and PDGF A and B has been demonstrated in both high- and low-grade human astrocytomas[15,37] as well as in oligodendrogliomas[16]. In human meningiomas, only PDGFRβ expression is increased[11,14].

In agreement with these findings, increased PDGFRα expression was not seen in any of the canine meningioma samples. The pattern of expression of PDGFRα in canine tumours was different from that seen with the other growth factor

receptors, in that increased expression was seen predominantly in oligodendrogliomas. The minimally increased expression of PDGFRα in these canine astrocytomas may indicate that either PDGFRα does not have a major role in canine astrocytomas compared to their human counterparts or signalling may be occurring predominantly via the β receptor.

c-Met expression

Increased expression of c-Met has been reported in human astrocytomas, particularly in high-grade tumours[17–19]. Limited data exists regarding meningiomas and oligodendrogliomas; however, c-Met has been demonstrated immunohistochemically in meningiomas in one study[17]. Increased expression of c-Met was not found in one study looking at ungraded oligodendrogliomas[19].

Expression of c-Met was relatively low in most of the canine tumour samples in this study compared to the increased expression seen with other growth factor tyrosine kinase receptors. Consistent increased expression was only seen in the grade III canine oligodendroglial tumours. The majority of human oligodendrogliomas are grade II tumours in contrast to the majority of grade III oligodendrogliomas in this study. This difference may explain the increased c-Met expression seen in oligodendrogliomas in this study compared to human oligodendrogliomas[19].

The relatively small sample size and selective sampling from individual tumours limit the conclusions that may be drawn from the data presented, particularly with respect to individual tumour samples. However, the results of this study provide evidence that increased expression of several major tyrosine kinase growth factor receptors is a common finding in canine primary brain tumours, particularly in high-grade gliomas.

Canine primary tumours have striking similarities to their human counterparts in terms of biological behaviour, imaging and histological characteristics. Increased expression of receptor tyrosine kinases in canine brain tumours suggests that similarities may also be present at the molecular level. This finding validates the use of growth-factor-targeted therapies as a rational treatment strategy in dogs and supports the use of spontaneous canine primary brain tumours as a model system for human disease.

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References

1. Machein MR and Plate KH. VEGF in brain tumors. *Journal of Neuro-Oncology* 2000; **50:** 109–120.
2. Yao Y, Kubota T, Sato K, Kitai R, Takeuchi H and Arishima H. Prognostic value of vascular endothelial growth factor and its receptors Flt-1 and Flk-1 in astrocytic tumours. *Acta Neurochirurgica* 2001; **143:** 159–166.
3. Hatva E, Kaipainen A, Mentula P, Jaaskelainen J, Paetau A, Haltia M and Alitalo K. Expression of endothelial cell-specific receptor tyrosine kinases and growth factors in human brain tumors. *American Journal of Pathology* 1995; **146:** 368–378.
4. Plate KH, Breier G, Weich HA, Mennel HD and Risau W. Vascular endothelial growth factor and glioma angiogenesis: coordinate induction of VEGF receptors, distribution of VEGF protein and possible in vivo regulatory mechanisms. *International Journal of Cancer* 1994; **59:** 520–529.
5. Chan AS, Leung SY, Wong MP, Yuen ST, Cheung N, Fan YW and Chung LP. Expression of vascular endothelial growth factor and its receptors in the anaplastic progression of astrocytoma, oligodendroglioma, and ependymoma. *American Journal of Surgical Pathology* 1998; **22:** 816–826.
6. Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P and Ohgaki H. Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. *Brain Pathology* 1996; **6:** 217–223; discussion 223–214.
7. Libermann TA, Razon N, Bartal AD, Yarden Y, Schlessinger J and Soreq H. Expression of epidermal growth factor receptors in human brain tumors. *Cancer Research* 1984; **44:** 753–760.
8. Ekstrand AJ, James CD, Cavenee WK, Seliger B, Pettersson RF and Collins VP. Genes for epidermal growth factor receptor, transforming growth factor alpha, and epidermal growth factor and their

expression in human gliomas in vivo. *Cancer Research* 1991; **51:** 2164–2172.
9. Reifenberger G, Prior R, Deckert M, Wechsler W. Epidermal growth factor receptor expression and growth fraction in human tumours of the nervous system. *Virchows Archive A Pathological Anatomy and Histopathology* 1989; **414:** 147–155.
10. Reifenberger J, Reifenberger G, Ichimura K, Schmidt EE, Wechsler W and Collins VP. Epidermal growth factor receptor expression in oligodendroglial tumors. *American Journal of Pathology* 1996; **149:** 29–35.
11. Kuratsu JI, Seto H, Kochi M and Ushio Y. Expression of PDGF, PDGF-receptor, EGF-receptor and sex hormone receptors on meningioma. *Acta Neurochirurgica* 1994; **131:** 289–293.
12. Jones NR, Rossi ML, Gregoriou M and Hughes JT. Epidermal growth factor receptor expression in 72 meningiomas. *Cancer* 1990; **66:** 152–155.
13. Carroll RS, Black PM, Zhang J, Kirsch M, Percec I, Lau N and Guha A. Expression and activation of epidermal growth factor receptors in meningiomas. *Journal of Neurosurgery* 1997; **87:** 315–323.
14. Black PM, Carroll R, Glowacka D, Riley K and Dashner K. Platelet-derived growth factor expression and stimulation in human meningiomas. *Journal of Neurosurgery* 1994; **81:** 388–393.
15. Guha A, Dashner K, Black PM, Wagner JA and Stiles CD. Expression of PDGF and PDGF receptors in human astrocytoma operation specimens supports the existence of an autocrine loop. *International Journal of Cancer* 1995; **60:** 168–173.
16. Di Rocco F, Carroll RS, Zhang J and Black PM. Platelet-derived growth factor and its receptor expression in human oligodendrogliomas. *Neurosurgery* 1998; **42:** 341–346.
17. Moriyama T, Kataoka H, Kawano H, Yokogami K, Nakano S, Goya T, Uchino H, Koono M and Wakisaka S. Comparative analysis of expression of hepatocyte growth factor and its receptor, c-met, in gliomas, meningiomas and schwannomas in humans. *Cancer Letters* 1998; **124:** 149–155.
18. Koochekpour S, Jeffers M, Rulong S, Taylor G, Klineberg E, Hudson EA, Resau JH and Vande Woude GF. Met and hepatocyte growth factor/scatter factor expression in human gliomas. *Cancer Research* 1997; **57:** 5391–5398.
19. Hirose Y, Kojima M, Sagoh M, Hayashi T, Murakami H, Shimazaki K, Yoshida K and Kawase T. Clinical importance of c-Met protein expression in high grade astrocytic tumors. *Neurologia Medico-Chirurgica* 1998; **38:** 851–858; discussion 858–859.
20. Hayes HM, Priester WA Jr and Pendergrass TW. Occurrence of nervous-tissue tumors in cattle, horses, cats and dogs. *International Journal of Cancer* 1975; **15:** 39–47.
21. Koestner A, Bilzer T, Fatzer R, Schulman FY, Summers BA and Van Winkle TJ. *Histological Classification of Tumors of the Nervous System of Domestic Animals*, 2nd edn., Washington, D.C., The Armed Forces Institute of Pathology, 199**9:** 71.
22. Palmer AC. Tumors of the central nervous system. *Proceedings of the Royal Society of Medicine* 1976; **69:** 49–51.
23. Bagley RS, Gavin PR, Moore MP, Silver GM, Harrington ML and Connors RL. Clinical signs associated with brain tumors in dogs: 97 cases (1992–1997). *Journal of the American Veterinary Medical Association* 1999; **215:** 818–819.
24. Kraft SL, Gavin PR, DeHaan C, Moore M, Wendling LR and Leathers CW. Retrospective review of 50 canine intracranial tumors evaluated by magnetic resonance imaging. *Journal of Veterinary Internal Medicine* 1997; **11:** 218–225.
25. Vandevelde M. Brain tumors in domestic animals: an overview. In: *Proceedings from the Conference on Brain Tumors in Man and Animals*, Research Triangle Park, North Carolina, 1984.
26. Priester WA and Mantel N. Occurrence of tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. *Journal of the National Cancer Institute* 1971; **47:** 1333–1344.
27. Summers BA, Cummings JF and de Lahunta A. Tumors of the central nervous system. In: *Veterinary Neuropathology*, St Louis, Mosby, 199**5:** 351–401.
28. Heidner GL, Kornegay JN, Page RL, Dodge RK and Thrall DE. Analysis of survival in a retrospective study of 86 dogs with brain tumors. *Journal of Veterinary Internal Medicine* 1991; **5:** 219–226.
29. Foster ES, Carrillo JM and Patnaik AK. Clinical signs of tumors affecting the rostral cerebrum in 43 dogs. *Journal of Veterinary Internal Medicine* 1988; **2:** 71–74.
30. Lipsitz D, Higgins RJ, Kortz GD, Dickinson PJ, Bollen AW and LeCouteur RA. Glioblastoma multiforme: clinical findings, magnetic resonance imaging and pathology in 5 dogs. *Veterinary Pathology* 2003; **40:** 659–669.
31. Stoica G, Kim HT, Hall DG and Coates JR. Morphology, immunohistochemistry, and genetic alterations in dog astrocytomas. *Veterinary Pathology* 2004; **41:** 10–19.
32. Berens ME, Bjotvedt G, Levesque DC, Rief MD, Shapiro JR and Coons SW. Tumorigenic, invasive,

karyotypic, and immunocytochemical characteristics of clonal cell lines derived from a spontaneous canine anaplastic astrocytoma. *In Vitro Cellular & Developmental Biology. Animal* 1993; **29A**: 310–318.
33. Kleihues P, Louis DN, Scheithauer BW, Rorke LB, Reifenberger G, Burger PC and Cavenee WK. The WHO classification of tumors of the nervous system. *Journal of Neuropathology and Experimental Neurology* 2002; **61:** 215–225; discussion 226–219.
34. Perry A, Scheithauer BW, Stafford SL, Lohse CM and Wollan PC. "Malignancy" in meningiomas: a clinicopathologic study of 116 patients, with grading implications. *Cancer* 1999; **85:** 2046–2056.
35. Foley JE, Rand C and Leutenegger C. Inflammation and changes in cytokine levels in neurological feline infectious peritonitis. *Journal of Feline Medicine and Surgery* 2003; **5:** 313–322.
36. Leutenegger CM, Mislin CN, Sigrist B, Ehrengruber MU, Hofmann-Lehmann R and Lutz H. Quantitative real-time PCR for the measurement of feline cytokine mRNA. *Veterinary Immunology and Immunopathology* 1999; **71:** 291–305.
37. Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B and Nister M. Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. *Cancer Research* 1992; **52:** 3213–3219.
38. Morokoff AP and Novak U. Targeted therapy for malignant gliomas. *Journal of Clinical Neuroscience* 2004; **11:** 807–818.
39. Restucci B, Borzacchiello G, Maiolino P, Martano M, Paciello O and Papparella S. Expression of vascular endothelial growth factor receptor Flk-1 in canine mammary tumours. *Journal of Comparative Pathology* 2004; **130:** 99–104.
40. Rawlings NG, Simko E, Bebchuk T, Caldwell SJ and Singh B. Localization of integrin alpha(v)beta3 and vascular endothelial growth factor receptor-2 (KDR/Flk-1) in cutaneous and oral melanomas of dog. *Histology and Histopathology* 2003; **18:** 819–826.
41. Scheidegger P, Weiglhofer W, Suarez S, Kaser-Hotz B, Steiner R, Ballmer-Hofer K and Jaussi R. Vascular endothelial growth factor (VEGF) and its receptors in tumor-bearing dogs. *Biological Chemistry* 1999; **380:** 1449–1454.
42. Donnay I, Devleeschouwer N, Wouters-Ballman P, Leclercq G and Verstegen J. Relationship between receptors for epidermal growth factor and steroid hormones in normal, dysplastic and neoplastic canine mammary tissues. *Research in Veterinary Science* 1996; **60:** 251–254.
43. Rutteman GR, Foekens JA, Portengen H, Vos JH, Blankenstein MA, Teske E, Cornelisse CJ and Misdorp W. Expression of epidermal growth factor receptor (EGFR) in non-affected and tumorous mammary tissue of female dogs. *Breast Cancer Research and Treatment* 1994; **30:** 139–146.
44. Nerurkar VR, Seshadri R, Mulherkar R, Ishwad CS, Lalitha VS and Naik SN. Receptors for epidermal growth factor and estradiol in canine mammary tumors. *International Journal of Cancer* 1987; **40:** 230–232.
45. Ferracini R, Angelini P, Cagliero E, Linari A, Martano M, Wunder J and Buracco P. MET oncogene aberrant expression in canine osteosarcoma. *Journal of Orthopaedic Research* 2000; **18:** 253–256.
46. Herold-Mende C, Steiner HH, Andl T, Riede D, Buttler A, Reisser C, Fusenig NE and Mueller MM. Expression and functional significance of vascular endothelial growth factor receptors in human tumor cells. *Laboratory Investigation* 1999; **79:** 1573–1582.
47. Shibuya M. Structure and dual function of vascular endothelial growth factor receptor-1 (Flt-1). *International Journal of Biochemistry & Cell Biology* 2001; **33:** 409–420.