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Telomerase reverse transcriptase (TERT) expression and proliferation in canine brain tumours

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Telomerase is a ribonucleoprotein enzyme complex that synthesizes telomere DNA. It is detected in 85–90% of malignant tumours in humans, but not in most somatic cells. Because telomerase plays a critical role in cell immortality, it represents an important target for anticancer therapies. We have previously shown that the dog is a potentially useful model for evaluating telomerase-based therapeutics. In this present study we analysed 93 canine brain tumours for telomerase reverse transcriptase (TERT) expression by immunohistochemistry. TERT immunoreactivity was detected in 16 of 50 grade 1 (32%) and 29 of 43 grade 2 tumours (67.4%), demonstrating a statistically significant association with histological grade ($P = 0.00012$). A subset of 51 tumours was also assessed for MIB-1 expression. The MIB-1 labelling index (LI) was found to correlate significantly with tumour grade, with a mean MIB-1 LI of 1.5% for grade 1 tumours, as compared with a mean MIB-1 LI of 21.7% for grade 2 tumours ($P << 0.001$). The MIB-1 LI was also significantly associated with TERT expression in all brain tumours ($P << 0.001$). These data further support the dog as a model for the preclinical development of telomerase-based therapeutics in brain tumours.

Keywords: brain tumours, dog, immunohistochemistry, Ki67, telomerase, TERT

Introduction

Telomerase is a cellular reverse transcriptase that adds nucleotides to the ends of telomeres, thereby preventing telomeric shortening and the onset of cellular senescence that occurs with the accumulation of cell divisions [1]. Activity is essential for embryogenesis but is repressed upon tissue differentiation during development such that telomerase is absent from birth in most somatic tissues [2]. Some cell types, however, including male germ cells, activated lymphocytes and stem cell populations continue to express telomerase at reduced levels [3–5]. In contrast, 85–90% of human cancers possess telomerase activity (TA) [6,7]. The enzyme telomerase is composed of an RNA component (TR), a catalytic subunit [(telomerase reverse transcriptase (TERT)] and associated proteins [8,9]. The TERT component is considered the primary determinant

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for activity for the following reasons: (1) TR is present in all tissues irrespective of TA [10]; (2) human TERT (hTERT) activity is repressed in normal somatic tissues, but expression is elevated in the majority of human tumours and in stem cells, correlating with TA [6]; (3) exogenous expression of hTERT in telomerase-negative normal human cells *in vitro* is associated with the presence of TA and with the extension of cellular lifespan [11,12] and (4) the inhibition of hTERT expression represses TA and limits the lifespan of cancer cells [13].

Several studies have demonstrated that hTERT is a potentially useful diagnostic and prognostic marker for cancer [14,15] as well as being an ideal target for therapy. To date the most successful telomerase-based therapeutic approaches that have been evaluated in preclinical trails have utilized the cancer specificity of telomerase gene expression to develop gene therapy strategies [16–18]. However, problems exist in taking these preclinical trials further, due to the lack of a suitable animal model. In mice, TA is present in all adult tissues and the telomerase subunits are not as tightly regulated as in human tissues [19,20]. Therefore the effects of tumour targeting therapies on normal tissues will probably be difficult to evaluate in mouse models. The presence of TA in canine tumour tissues has been investigated and these studies have analysed over 100 canine solid tumours demonstrating that more than 95% of canine cancers examined to date are associated with TA [21]. We have previously shown that TA in dogs is confined to tumour tissues and cells with a high proliferative potential (e.g. testis, lymphoid tissue) with little or no activity in normal somatic tissues [22]. Similarly, we have demonstrated that TA can be detected in immortal canine tumour cell lines and show that primary fibroblasts undergo telomeric attrition [22]. More recently, we have also shown that the dog TERT promoter is similar to the human promoter in terms of structure and tissue-specific activity [23]. Unlike other model systems, canine tumours arise spontaneously in a naturally outbred population. Many canine tumours share multiple histopathological features with human tumours and their biological behaviour in response to radiotherapy and chemotherapy is often similar [24]. These similarities not only suggest that TERT may be of diagnostic importance in canine malignancies but also provide support for the dog as a useful animal model for *in vivo* preclinical testing of novel therapies.

In particular, naturally occurring canine brain tumours occur with a frequency approximately five times greater than those in humans [25] with a reported incidence of 14.5 tumours per 100 000 dogs compared with approximately 3–4 per 100 000 people [26,27]. The most common types of primary brain tumours in dogs are meningiomas, comprising between 30% and 39% of all intracranial neoplasms [28,29], and glial tumours (astrocytomas and oligodendrogliomas). Pituitary tumours, choroid plexus papillomas and ependymomas are also commonly described. The majority of brain tumour types described in humans have been reported in dogs, and have shown to be both histopathologically and clinically similar to their human counterparts [26–30]. The aim of this study was to evaluate the presence of TERT protein expression in a range of formalin-fixed, paraffin-embedded canine brain tumour samples and to compare expression with proliferation as assessed by the MIB-1 labelling index (LI), to determine its usefulness as a diagnostic tool in canine brain tumours and to evaluate whether canine brain tumours provide a useful preclinical model for the evaluation of telomerase-based therapeutics.

Materials and methods

Cell cultures

CMT7 (kindly donated by Prof Hellmann, University of Uppsala) and D17 (ATTC: CRL-6248) canine cell lines were grown in DMEM (Invitrogen, Paisley, UK) supplemented with 10% foetal calf serum. In house cultured CSF-01 cells (canine primary skin fibroblasts) were grown in MEM-α (Invitrogen, Paisley, UK) supplemented with 10% foetal calf serum. The presence of TA in the CMT7 and D17 cells have previously been reported [22].

Tissue samples

Ninety-three formalin-fixed samples of brain tumours (Table 1) were retrieved from the pathology archives of the Institute for Comparative Medicine, University of Glasgow Veterinary School and collaborating Institutes, namely, Institute of Animal Neurology, University of Berne; College of Veterinary Medicine, North Carolina State University; Department of Pathobiology, Faculty of Veterinary Medicine Utrecht University; Centre for Small Animal Studies, Animal Health Trust, UK. All sections used in the study were reevaluated by a pathologist and the diagnosis

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Table 1. Correlation between telomerase reverse transcriptase (TERT) immunopositivity, tumour grade and tumour subtype. *P*-values show significance of correlation between percentage of TERT-positive tumours and grading (grade 1 or 2)

Tumour type	*Grade*	*No.*	*No. TERT +ve*	*P-value*
Astrocytomas				
Astrocytoma	1	5	0	
Astrocytoma anaplastic	2	5	3	
Astrocytoma glioblastoma	2	4	2	
		14	5 (36%)	0.10
Meningiomas				
Meningioma	1	21	8	
Meningioma anaplastic	2	6	4	
		27	12 (44%)	0.008
Oligodendrogliomas				
Oligodendroglioma	1	17	7	
Oligodendroglioma anaplastic	2	10	8	
		27	15 (56%)	0.004
Choroid plexus tumours				
Choroid plexus papilloma	1	6	0	
Choroid plexus papilloma: anaplastic	2	1	1	
Choroid plexus papilloma: carcinoma	2	1	0	
		8	1 (13%)	0.19
Other				
Ependymoma	1	1	1	
Gliosarcoma	2	1	1	
Medulloblastoma	2	1	1	
Pituitary adenocarcinoma	2	1	1	
		4	4	N/A*
Metastases				
Adenocarcinoma	2	6	4	
Lymphoma	2	7	4	
		13	8 (61%)	N/A*
All tumours				
Grade 1		50	16	
Grade 2		43	29	
		93	45	0.00012

*Not applicable – insufficient group size (grade 1).

of intracranial neoplasia was confirmed. Each tumour sample was classified either as grade 1 or 2 using the World Health Organization classification system of tumours [30].

Immunohistochemistry

Paraffin-embedded samples were immunostained using a streptavidin–biotin complex. Paraffin sections were dewaxed in histo-clear (National Diagnostics, Atlanta, Georgia, USA), rehydrated in alcohol and incubated in 0.5% H_2O_2 methanol solution for 20 min. Cryostat sections were fixed in acetone before the quenching step. Paraffin sections were subjected to antigen retrieval with 0.01 M sodium citrate (pH 6.0) in a pressure cooker (75 s at 15 pounds per square inch). All sections were blocked in 1% normal unlabelled serum (Scottish Antibody Production Unit, Edinburgh, UK) in 0.01 M Tris-buffered saline for 30 min at room temperature and incubated for 2 h at room temperature with antibodies against hTERT (mouse monoclonal, BD Biosciences, diluted 1:200) and against Ki67 (MIB-1 mouse monoclonal, Dako, Ely, UK, 1:200 in 0.1% BSA). The sections were then incubated for 45 min with the appropriate biotinylated secondary antibody (DakoCytomation, Ely, UK) followed by horseradish peroxidase-conjugated streptavidin-biotin complex (DakoCytomation, Ely, UK) for 45 min. Immunoreactivity was visualized using diaminobenzidine (DAB) (Sigma, Gilling-

ham, UK) and sections counterstained with haematoxylin. Negative controls were run in parallel without the primary antibody step.

Evaluation of immunohistochemical staining

The immunohistochemical staining was evaluated by light microscopy. For TERT staining, a staining percentage of greater than 10% was regarded as positive. For each section the distribution of staining within cells and throughout each section was noted. To assess MIB-1 LI, a minimum of five fields were selected from areas with the highest staining and a minimum of 1000 nuclei per case counted, with the number of positive cells being expressed as a percentage of all nuclei counted. For comparison of MIB-1 LI and TERT staining with histological grade, the Mann–Whitney *U*-test was used, with the level of significance set at $P < 0.05$.

Telomerase activity

Telomerase activity was measured using the TeloTAGGG Telomerase PCR ELISA^{PLUS} assay (Roche Molecular Biochemicals, Lewes, UK). Briefly, cells were homogenized in 200 μl of ice-cold lysis buffer and incubated for 30 min on ice. After centrifugation at 16 000 **g** for 20 min at 4°C, the supernatant was collected, frozen in liquid nitrogen, and stored at −80°C. Protein concentrations were measured using the Bradford assay (Sigma, Gillingham UK). Protein samples were incubated with reaction buffer containing a biotin-labelled P1-TS primer and P2 primer, telomerase substrate, and *Taq* polymerase for 30 min at 25°C in a final volume of 50 μl. Internal standard (IS) were included in each reaction to control for the presence of PCR inhibitors in protein extracts. After a further incubation at 94°C for 5 min, the resulting mixture was subjected to PCR for 30 cycles of 30 s at 94°C, 30 s at 50°C, and 90 s at 72°C. The amplification products were denatured and hybridized with a digoxigenin-labelled, telomeric repeat-specific detection probe. The resulting product was immobilized through the biotin-labelled TS primer to a streptavidin-coated microtitre plate and detected with an antidigoxigenin antibody conjugated with horseradish peroxidase. Absorbance values were measured using a microtitre reader at 450 nm with a reference wavelength of 690 nm. Samples were regarded as telomerase positive if the absorbance was higher than 0.2 arbitrary units (A_{450nm}–A_{690nm}). The absorbance reading obtained with the positive control supplied with the kit was always higher than 2.0 U.

Results

Characterization of hTERT antibody cross reactivity in canine tissues

We tested three commercially available anti-hTERT antibodies. Staining was performed with hTERT antibodies supplied by Oncogene (Anti-TERT, which recognizes an internal 21-amino-acid sequence), Novocastra (NCL-hTERT, an antibody recognizing a 147-amino-acid sequence near the N terminal region of hTERT), and Alpha Diagnostics (EST-22A, an antibody directed against a 21-amino-acid sequence found in the mid-region of hTERT) in formalin-fixed and paraffin-embedded canine tissues. Only one antibody (NCL-hTERT; Novacastra, Newcastle-Upon-Tyne, UK) showed sufficient specificity in canine tissues and this antibody was used in all subsequent cross reactivity characterizations. To determine whether the anti-hTERT antibody (NCL-hTERT) could recognize canine TERT protein, TERT immunoreactivity was examined in D17 and CMT7 cells, and in canine testis tissue sections previously shown to be positive for TA [22]. Canine primary skin fibroblasts (CSF-01) cells negative for telomerase (data not shown) were used as negative controls. In both telomerase positive cell lines, TERT staining was restricted mainly to the nucleus (Figure 1**A**,**B**). Staining was particularly prominent in nucleoli, and in some cells, nucleolar staining was the only detectable pattern. In other cells, staining was distributed throughout the nucleus, either in a punctuate pattern or diffusely. Staining of the cytoplasm was also observed in some cells, although less intense than the nuclear staining. In normal canine testis, staining was confined to the germinal cells of the seminiferous tubules (Figure 1**C**). In contrast, no immunoreactivity was detected in the negative controls cells (Figure 1**D**).

TERT immunohistochemistry in canine brain tumours

To investigate expression of TERT in canine brain tumours, a total of 93 canine brain tumour sections were evaluated for TERT immunoreactivity using the NCL-hTERT antibody. The staining pattern observed varied between tumours, with most tumours exhibiting granular nuclear staining with distinct nucleolar staining; in a few

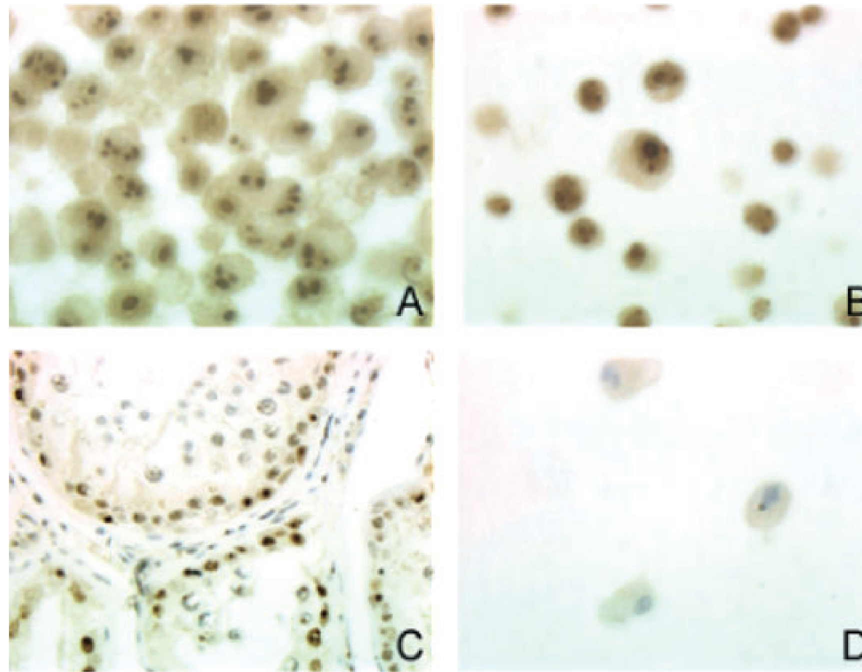

Figure 1. Telomerase reverse transcriptase (TERT) staining in canine cells and tissues. The presence of TERT protein is indicated by a brown signal. (**A**) TERT staining in canine telomerase positive D17 cells. Note strong nucleolar staining and fainter, more diffuse nucleoplasmic staining. (**B**) TERT staining in canine telomerase positive CMT7 cells. (**C**) TERT immunostaining localized to the germinal cells of the seminiferous tubules in canine testis CMT7 cells. (**D**) Negative TERT staining in canine primary fibroblasts. Magnification: **A**, **B**, **D**: ×400; **C**: ×100.

cases a more diffuse nuclear staining was seen. Representative examples of TERT staining observed are shown in Figure 2. Meningiomas (Figure 2**B**) and oligodendrogliomas (Figure 2**C**) tended to show a more diffuse staining pattern with the majority of cells within each tumour staining positive. In contrast, astrocytomas tended to exhibit a more patchy staining pattern, with some areas focally positive and the remainder of the tumour negative (Figure 2**D**). In some tumours staining was predominantly nucleolar (Figure 2**E**) while in others staining occurred more diffusely through the nucleus. In some glioblastomas, TERT staining was not restricted to tumour cells but was also found to be present in areas of vascular endothelial hyperplasia (Figure 2**F**). In these areas, endothelial cells stained positively, either with nucleolar staining or with a more diffuse nuclear localization. In addition, there appeared to be some background cytoplasmic staining in these cells. Faint background cytoplasmic staining was also found in choroid plexus papillomas which contain cells of endothelial origin.

As shown in Table 1, TERT immunoreactivity was detected in 45 of 93 (48%) brain tumour tissues consisting of 16 of 50 grade 1 (32%) and 29 of 43 grade 2 tumours (67.4%), demonstrating a statistically significant association with histological grade overall ($P = 0.00012$). When metastases were excluded, the association between TERT immunoreactivity and grade remained statistically significant ($P = 0.00014$). TERT immunoreactivity was demonstrated in all tumour subtypes and a statistically significant association between TERT staining and histological grade was observed for meningiomas ($P = 0.008$) and oligodendrogliomas ($P = 0.004$) (Table 1). TERT reactivity was observed least commonly in choroid plexus tumours where only one of eight tumours analysed was positive. The subgroup with the highest number of TERT-positive tumours were the metastatic tumours.

MIB-1 immunohistochemistry and correlation with TERT expression

Fifty-one of the canine brain tumours analysed for TERT immunopositivity were also evaluated for MIB-1. Immunoreactivity was exclusively confined to the nucleus with no evidence of cytoplasmic staining. Numerous strongly immunoreactive nuclei were found in all types of carcinoma cells (Figure 3). The MIB-1 LI was found to signifi-

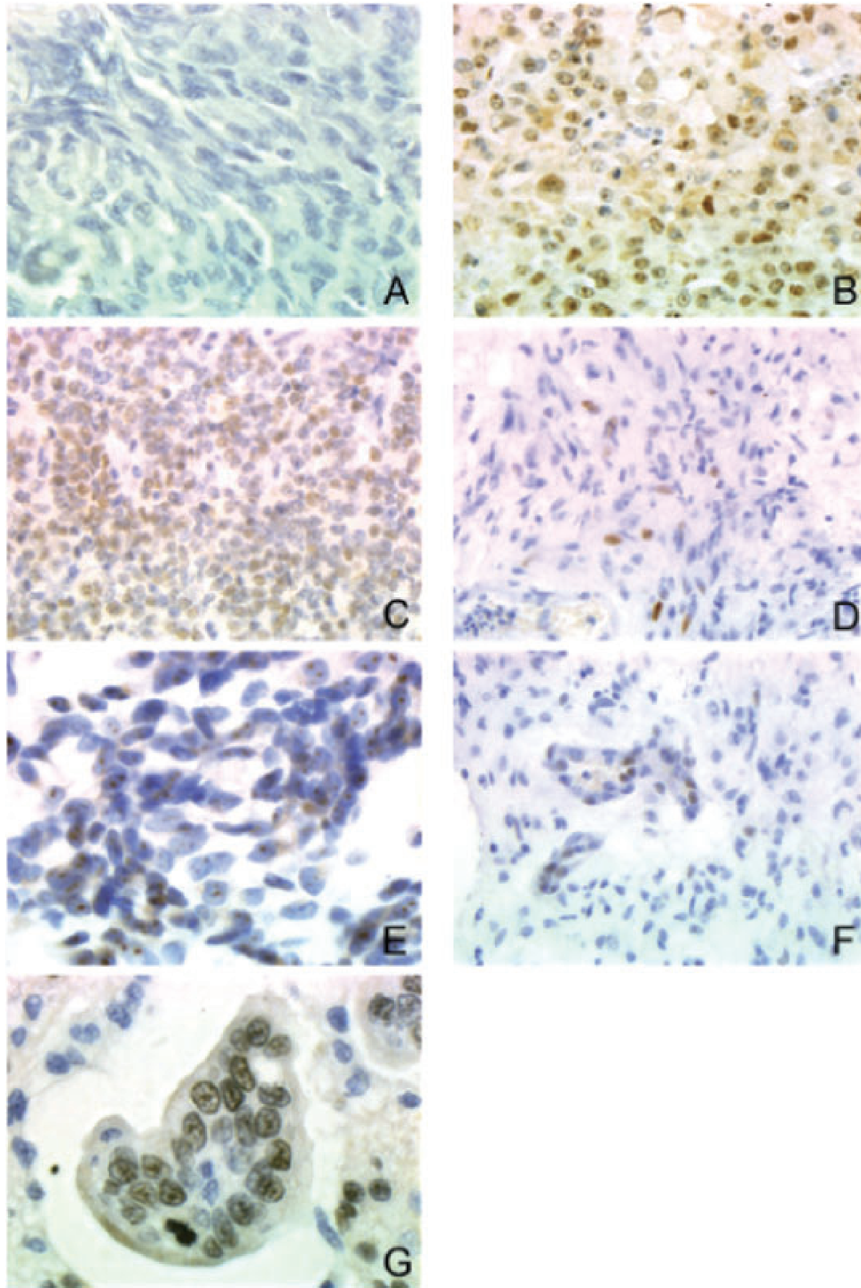

Figure 2. Telomerase reverse transcriptase (TERT) staining in canine brain tumours. (**A**) TERT-negative transitional meningioma. (**B**) TERT-positive anaplastic meningioma. (**C**) TERT-positive oligodendroglioma. Note diffuse distribution of positively stained cells. (**D**) TERT-positive anaplastic astrocytoma. Note majority of cells with predominant nucleolar staining. (**E**) TERT-positive glioblastoma. Note patchy distribution of positively stained cells with remainder of field TERT-negative. (**F**) Glioblastoma. Note positively staining vascular endothelial cells within a predominantly TERT-negative field. (**G**) Metastatic adenocarcinoma. Note single focus of metastatic cells within a TERT-negative section of normal brain. Magnification: **A**, **B**, **D**: ×200; **C**, **E**, **F**: ×100; **G**: ×400.

cantly associate with tumour grade, with a mean MIB-1 LI of 1.5% for grade 1 tumours, as compared with a mean MIB-1 LI of 21.7% for grade 2 tumours ($P << 0.001$) (Table 2). A significant association was also observed between MIB-1 LI and TERT expression in all brain tumours ($P << 0.001$) and in the meningioma and oligodendroglioma subtypes ($P = 0.002$ and $P = 0.007$ respectively) (Table 2).

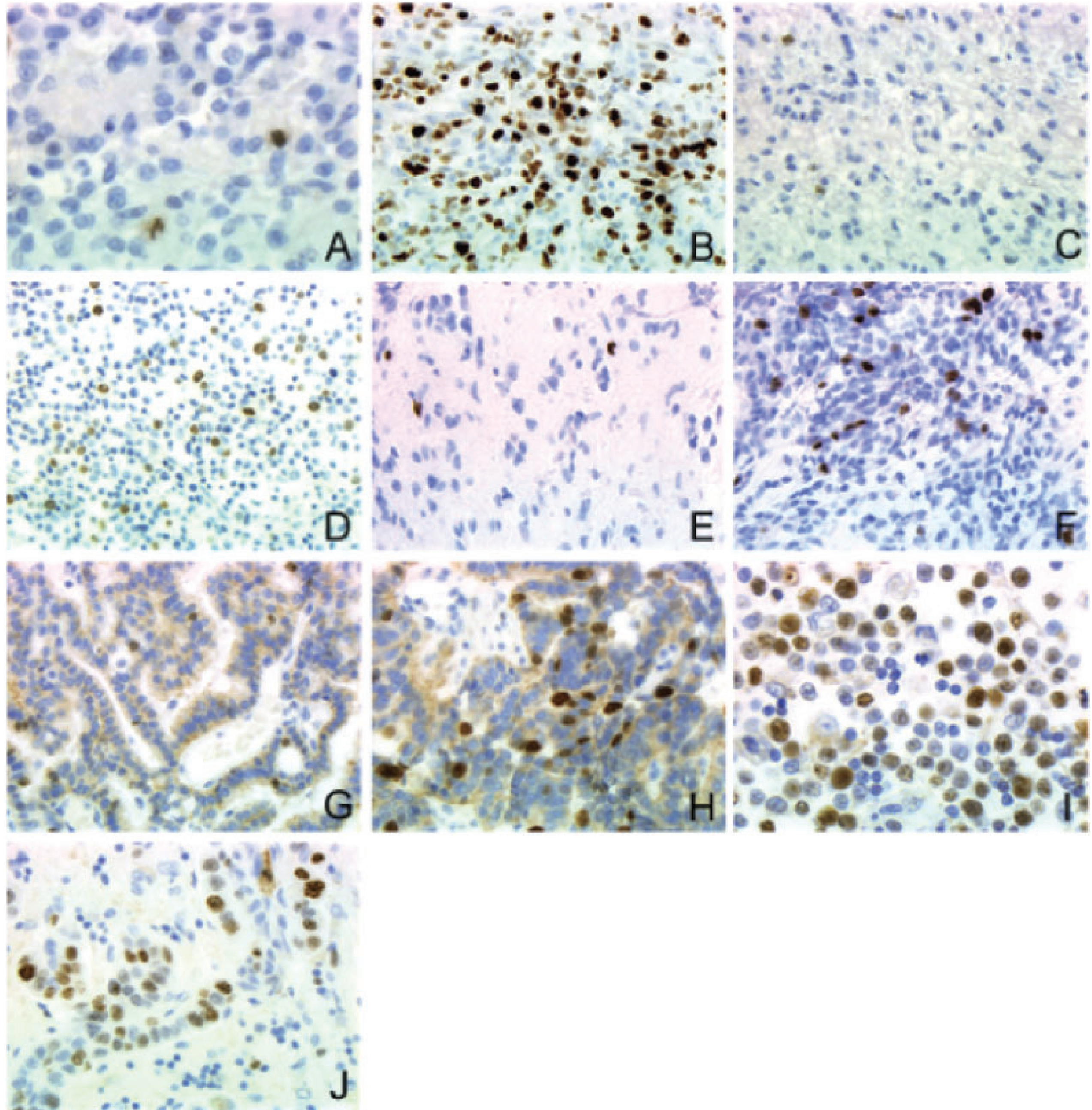

Figure 3. MIB-1 labelling index (LI) in canine brain tumours. (**A**) Meningioma (LI = 1.2) (×400). (**B**) Anaplastic meningioma (LI = 41.4) (×200). (**C**) Oligodendroglioma (LI = 2.8) (×200). (**D**) Anaplastic oligodendroglioma (LI = 13.4) (×100). (**E**) Astrocytoma (LI = 3.2) (×200). (**F**) Anaplastic astrocytoma (LI = 8.9) (×200). (**G**) Choroid plexus papilloma (LI = 3.0) (×200). (**H**) Choroid plexus carcinoma (LI = 9.0) (×200). (**I**) Lymphoma (LI = 49.3) (×400). (**J**) Metastatic carcinoma (LI = 10.4) (×200).

Discussion

We first characterized the antibody NCL-hTERT which recognizes a 147-amino-acid sequence near the N terminal region of hTERT, to determine cross reactivity with the canine TERT protein. Immunohistochemistry showed significant positive staining in canine cell lines previously reported to be positive for TA and the absence of staining in primary canine fibroblasts, previously shown to be TA negative [22]. Staining was also observed in normal

Table 2. Correlation between mean MIB-1 staining, telomerase reverse transcriptase (TERT) immunopositivity, tumour grade and subtype. *P*-values show significance of correlation between MIB-1 labelling index and TERT positivity

Tumour type	*Grade*	*No.*	*Mean MIB-1 LI (%)*	*MIB-1 range (%)*	*TERT +ve*	*P-value*
Astrocytomas						
Astrocytoma	1	1	3.2	3.2	0	
Astrocytoma anaplastic	2	2	11.6	8.9–14.2	1	
Astrocytoma glioblastoma	2	2	22.9	19.6–26.1	1	
		5			2	0.44
Meningiomas						
Meningioma	1	10	0.0	0–2.0	0	
Meningioma anaplastic	2	5	19.3	13.0–41.4	4	
		15			4	0.0015
Oligodendrogliomas						
Oligodendroglioma	1	10	2.5	0–8.9	2	
Oligodendroglioma anaplastic	2	9	22.3	9.2–37.8	8	
		19			10	0.007
Choroid plexus tumours						
Choroid plexus papilloma	1	5	0.8	0–0.3	0	
Choroid plexus papilloma: carcinoma	2	1	9.2	N/A	0	
		6			0	0.19
Other						
Gliosarcoma	2	1	26.1	N/A*	1	
Pituitary adenocarcinoma	2	1	16.9	N/A*	1	
		2			2	N/A†
Metastases						
Lymphoma	2	3	47.1	19.4–72.5	2	
Adenocarcinoma	2	1	19.4	N/A	0	
		4			2	N/A†
All tumours						
Grade 1		26	1.5	0–8.9	2	
Grade 2		25	21.7	8.9–72.5	18	
Total		51			20	<<0.001

*Not applicable – insufficient sample size. †Not applicable – insufficient group size (grade 1).

canine testis tissue positive for TA, and the signal specifically localized as expected to the germinal cells of the seminiferous tubules. The immunoreactivity staining pattern seen in canine tumour cells is similar to that described in humans; most of the staining is confined to the nucleus, in a granular or diffuse pattern, with strong nucleolar localization [17,31–34]. The strong nucleolar staining is thought to represent detection of the telomerase holoenzyme, which is assembled within the nucleolus [33]. The granular or 'speckled' appearance throughout the nucleus may represent the active telomerase complex at chromosome ends [34]. Some authors have attempted to differentiate between those tumours in which nucleolar staining alone predominates from those in which nuclear and nucleolar staining occur. However, there would appear to be little clinical significance attached to the particular type seen [35]. The staining of endothelial cells may reflect a nonspecific interaction with the antibody, as several other antibodies stain cell cytoplasm in a nonspecific manner. Alternatively, the cytoplasmic staining may represent shuttling of the telomerase holoenzyme from the nucleus out to the cytoplasm and then back into the nucleus during the assembly process [35].

In addition the TERT staining patterns seen in human brain tumours are mirrored in canine brain tumours. In particular the more homogenous genetic make-up of oligodendroglial tumours appears to be reflected in the TERT staining pattern, with most TERT-positive oligodendrogliomas showing positive staining in the vast majority of cells diffusely throughout the tumour (Figure 2) [36]. The trend towards more focal, patchy TERT staining in astrocytomas also appears to parallel expression in human tumours [36]. This may reflect the evolution of these different tumour types. The fact that glioblastoma multi-

forme may arise through one of several different pathways, either as a *de novo* tumour or as malignant transformation of more benign astrocytoma subtypes (secondary glioblastoma), demonstrates the genetic variation underlying this phenotype [37]. Some authors have suggested that telomerase activation occurs early in glioblastomas, and that with increasing tumour growth and the development of necrosis, TA subsequently disappears in some areas [38]. However, other authors have suggested that telomerase activation in these tumours occurs in areas of greater anaplasia, a finding which would support telomerase activation at a late stage in tumour development as anaplasia is more likely to occur later in tumour development [36]. Whether this pattern of telomerase loss with glioblastoma development occurs in dogs as well as in humans, however, remains to be investigated.

The finding that TERT is present in endothelial cells undergoing vascular endothelial hyperplasia has also been reported in humans [39,40]. The involvement of telomerase in tumour angiogenesis is likely to be the result of similar factors responsible for tumour development. For instance, the SP-1 transcription factor which is known to be important for the activation of TERT transcription, also acts on the promoter region of the Vascular Endothelial Growth Factor gene, a major player in the initiation of angiogenesis in glioblastomas as well as in other pathological situations [39].

In the present study, a MIB-1 LI of approximately 9% was found to be the cut-off between grade 1 and grade 2 tumours. In general, the majority of grade 1 tumours had a low MIB-1 LI, usually less than 4%. One interesting finding was that two of the grade 2 meningiomas had a MIB-1 LI of >20%; however, three had a substantially lower MIB-1 of between 9% and 14%. In humans, the WHO classification includes three grades of meningioma: the benign, atypical and anaplastic variants. Of these, the atypical variant has been reported to have a MIB-1 LI of less than 20% while the anaplastic variant has a MIB-1 LI of greater than 20% [41]. In dogs, the current WHO classification describes only two variants: benign and anaplastic. Our results suggest that in dogs a third variant may be present based on a proliferative index of greater than 9% but less than 20%, as described in humans. Further work examining a larger group of meningiomas and correlating survival with grading would be needed to confirm this. Interestingly, the tumour group with the highest MIB-1 LI was the metastatic tumour group, especially the lymphomas.

In the present study, TERT staining was associated with MIB-1 staining in the brain tumours assessed as a single group, as well as in the meningioma and oligodendroglioma subgroups. The association between MIB-1 staining and TA in human brain tumours is not entirely clear. While studies have found that TA is associated with proliferation, some studies have not [42]. Given that increased proliferation is the most useful prognostic indicator for many brain tumour types, and that TA is commonly associated with malignancy, it would be expected that these two characteristic features of tumours should overlap to some degree. However, it is not entirely clear whether the two factors are directly linked or whether they stem from a common determinant of tumour behaviour. It has been suggested that the vast majority of breast tumour cells undergoing proliferation also express telomerase, suggesting that at least in some tumour types the pathways initiating these two factors are shared by the same cells [14]. However, it is possible that telomerase activation may occur either before or after the signal to proliferate has been given. For instance, Maes *et al*. [35] found that TERT expression could be found in a subset of benign meningiomas, and that this predicted survival where proliferative index did not. This suggests that at least in these tumours TERT expression occurs before significant proliferation. Although in canine brain tumours overall we found a direct relationship between TERT expression and cellular proliferation, significant variation was found between the different tumour subtypes. For instance, one case of metastatic lymphoma exhibited the highest MIB-1 LI of 72.5%, was found not to express TERT. In humans, the level of telomerase expression in metastatic brain tumours does not seem to correlate with either the tumour type or patient survival, although the majority of metastatic tumours do possess TA [43]. Our study appears to show a similar situation, with three out of four of the metastatic tumours examined being positive for TERT expression.

There is considerable evidence already from the human literature that TERT expression will indeed be a useful prognostic indicator. For example, in meningiomas, up to 20% of the most benign subtype will recur despite surgical resection. While proliferative markers do not predict those tumours which go on to recur, Maes *et al*. [35] found that TERT expression does, as recurrent tumours were found to have significantly more cells TERT-positive than nonrecurrent tumours. Simon *et al*. [44] reported similar findings, finding that all recurrent meningiomas in their study had

acquired telomerase expression. Similarly, Harada *et al*. [45] found a similar situation in tracking a recurrent pituitary tumour, with successive resections of the same tumour showing an increasing level of TA. Studies examining TA within individual tumours as they progress are likely to provide useful information on tumour progression which is not necessarily available from surveying larger groups of tumours.

While our understanding of the biology of cancers has increased rapidly due to advances in molecular biology and genetics, such advances have not translated to the clinical setting at the same pace, largely because of the lack of preclinical *in vivo* model systems. Genetically engineered mice are excellent cancer models for translational research, but few have been involved in the preclinical development of novel therapeutics [46]. Telomerase is clearly a target for therapeutic intervention; however, in mice telomerase is present in all adult tissues, the telomerase subunits are not as tightly regulated as in human tissues [19,20] and true tumour targeting will probably be difficult to demonstrate in mouse models. Dogs, however, represent useful animal models for cancer therapeutics; cancer in dogs is one of the major causes of death and occurs at a prevalence sufficient to be appropriate for preclinical trails. In the UK canine cancers occur at an estimated incidence of 1437 per 100 000 dogs per year [47] and in the USA approximately 55 million dogs are estimated to be at risk of developing cancer [24]. We have previously shown that TA (in terms of its tissue specific distribution) is similar in dogs as in humans and telomere lengths are also comparable in size [22]. Further, we have recently demonstrated that the canine TERT promoter is also similar to the human promoter in structure and activity [23]. The present study demonstrates that TERT expression shows strong similarities in canine brain tumours to human tumours and this datum is not only important in terms of diagnostics in canine brain malignancies but also further supports the dog as a preclinical model system for telomerase-based therapeutics.

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