PDGFs and PDGFRs in canine osteosarcoma: New targets for innovative therapeutic strategies in comparative oncology

Lorella Maniscalco, Selina Iussich, Emanuela Morello, Marina Martano, Bartolomeo Biolattia, Fulvio Riondato, Leonardo Della Salda, Mariarita Romanucci, Daniela Malatesta, Laura Bongiovanni, Federica Tirritoa, Francesca Gattinoa, Paolo Buracco, Raffaella De Maria

ARTICLE INFO

Article history:
Accepted 7 May 2012

Keywords:
PDGF
PDGFR receptor
Canine osteosarcoma
Comparative oncology
Targeted therapy

A B S T R A C T

Platelet derived growth factor receptor (PDGFR)α and PDGFRβ are tyrosine kinase receptors that are over-expressed in 70–80% of human osteosarcomas (OSAs) and may be suitable therapeutic targets for specific kinase inhibitors (TKIs). Canine OSA shows histopathological and clinical features similar to human OSA, and is considered an excellent model in comparative oncology. This study investigated PDGFA, PDGF-B, PDGFRα and PDGFRβ expression in 33 canine OSA samples by immunohistochemistry and in seven primary canine OSA cell lines by Western blot and quantitative PCR analysis. Immunohistochemical data showed that PDGFA and PDGF-B are expressed in 42% and 60% of the OSAs analysed, respectively, while PDGFRα and PDGFRβ were expressed in 78% and 81% of cases, respectively. Quantitative PCR data showed that all canine OSA cell lines overexpressed PDGFRα, while 6/7 overexpressed PDGFRβ and PDGFA relative to a normal osteoblastic cell line. Moreover, in vitro treatment with a specific PDGFR inhibitor, AG1296, caused a dose- and time-dependent decrease in AKT phosphorylation. Collectively, these data show that PDGFRs/PDGFs are co-expressed in canine osteosarcomas, which suggests that an autocrine and/or paracrine loop is involved and that they play an important role in the aetiology of OSA. PDGFRs may be suitable targets for the treatment of canine OSA with a specific TKI.

© 2012 Elsevier Ltd. All rights reserved.

Introduction

Spontaneous tumours in dogs and cats are suitable models of human cancer (Withrow et al., 1991; Vail and MacEwen, 2000; Khanna et al., 2006). Canine osteosarcoma (OSA), in particular, represents an excellent model for OSA in humans due to the relatively high incidence of the tumour in dogs, similarities in the biological behaviour of the disease between the two species, common molecular features, the large body size of the breeds that are frequently affected and the similar physical environments of the disease (Morello et al., 2011). In dogs, OSA represents the most common primary malignant tumour of bone (80% of all bone tumours) and is characterised by a locally aggressive and highly metastatic behaviour, mainly in large and giant breeds of dogs (Farese et al., 2009).

Similarities between dog and human OSA also exist at the molecular level: canine OSA cell lines and tumours, like their human counterparts (Freeman et al., 2008; Kaseta et al., 2008; Wu et al., 2011), frequently contain mutations that inactivate p53 (Johnson et al., 1998) and the PTEN family of tumour suppressor genes (Levine et al., 2002). Insulin and hepatocyte growth factor (HGF) influence tumour growth, invasion and malignant phenotype in canine and human OSA cell lines and tissues (Corlik et al., 1999; MacEwen et al., 2003, 2004; Ferrari et al., 2004). Overexpression of the erb-B2 gene, which encodes human epidermal growth factor receptor 2 (HER2), was observed in 86% and 40% of canine OSA cell lines and tissue samples, respectively (Flint et al., 2004).

Matrix metalloproteinases (MMPs) 2 and 9 and STAT3 are also expressed in canine and human OSA cell lines and tissues (Lana et al., 2000; Loukopoulos et al., 2004; Chen et al., 2007; Fossey et al., 2009; Korpi et al., 2011). Furthermore, we have recently demonstrated that (as in humans) 79% of canine OSA samples overexpress the MET oncogene and that cell motility and invasiveness are MET-dependent, as they are abrogated by small interfering RNAs that are specific for MET (De Maria et al., 2009). These similarities between canine and human OSA suggest that the canine system could be a suitable model to study novel therapeutic approaches for humans (Ma et al., 2003).

Platelet-derived growth factors (PDGFs) play important roles during wound healing and embryonic development. Their expression has been linked to several diseases, including cancer, in which...
these factors promote angiogenesis and autocrine stimulation of tumour cells (Alvarez et al., 2006). PDGFRα and PDGFRβ are tyrosine kinase receptors that can activate many of the same major signal transduction pathways including the PI3K (phosphatidylinositol 3-kinase), Ras mitogen-activated protein kinase (MAPK), phospholipase Cγ pathways and p-AKT (Liu et al., 2011). AKT and MAPK are cytoplasmic proteins kinases activated by phosphorylation (p-AKT and p-MAPK) that play an important role in cell survival, proliferation and cell growth by controlling the expression of anti-apoptotic genes (Seger and Krebs, 1995; Nicholson and Anderson, 2002; Tokunaga et al., 2008).

PDGFRs and PDGFRs are known to play a crucial role in the pathogenesis, invasion and distant metastasis of human cancers, and recent studies have suggested their involvement in an autocrine or paracrine loop that causes tumour growth and progression in OSA (Sulzbacher et al., 2000; Uren et al., 2003; Östman, 2004). In human OSA, the expression of PDGFRs does not appear to have a prognostic value, but they have been suggested as a therapeutic target for tyrosine kinase inhibitors (TKIs) (Sulzbacher et al., 2003, 2010; Kubo et al., 2008). In veterinary oncology, PDGFRs have been investigated only in spontaneous canine astrocytomas, where increased PDGFRα expression was observed (Higgins et al., 2010).

Dysfunction of tyrosine kinases occurs frequently in human cancers, and recent studies have indicated that a similar pattern of dysfunction is observed in canine and feline cancers (Lachowicz et al., 2005; London, 2009). TKIs that are specific for the c-KIT receptor and others are currently used in the treatment of canine mast cell tumours with excellent results (London et al., 2009). Despite these results, further molecular characterisation is needed to test the biological activity of TKIs in canine cancers and to investigate the effects of combining TKIs with standard therapeutics such as radiation therapy and chemotherapy. The main goal of this study was to evaluate the expression of PDGFRs and PDGFRs in canine OSAs and to demonstrate their biological role in tumour progression.

Materials and methods

Sample collection and clinical follow-up

Tissue samples were examined from 33 cases of spontaneous canine appendicular OSA treated between 2005 and 2010 at the Department of Animal Pathology of the University of Turin. None of the dogs included in this study had evidence of macroscopic metastases at presentation, and appendicular OSA was confirmed by histological diagnosis. In all cases, the initial data collected included history, a physical examination, complete blood count, serum biochemical profile, urinalysis and abdominal ultrasound. Limb (lateral–lateral [LL] and anterior–posterior [AP] views) radiographic evaluation was performed to examine the features and the extent of the tumour and the presence of lung metastasis at presentation, and appendicular OSA was confirmed by cytological features and by the lack of the expression of Ki67, p53 and PCNA evaluated by immunocytochemistry (data not shown).

Isolation and characterisation of primary canine osteosarcoma cell lines

Immediately following surgery, specimens of canine OSA and normal canine bone were collected in culture medium, washed in sterile phosphate buffered saline (PBS) and minced into small fragments (<1 mm³) in a sterile environment. Tissue fragments were then digested at 37 °C for 30–60 min in PBS containing 0.25 mg/ml collagenase type IA (Sigma–Aldrich). After digestion, the lysate was centrifuged at 184 for 1 min and the resulting pellet was suspended in Iscove’s standard medium supplemented with 10% foetal bovine serum (FBS), 1% glucose, 100 μg/ml penicillin and 100 μg/ml streptomycin. Cells were plated in 25 cm² tissue culture flasks, were then cultured at 37 °C in a humidified atmosphere of 5% CO₂ and the medium was renewed twice weekly.

To characterise the primary OSA cell lines, each line was tested for alkaline phosphatase activity. Cells grown in 6-well chamber slides were fixed in PBS containing 0.5% glutaraldehyde, washed twice in PBS and incubated overnight with NBT/BCIP solution (Sigma–Aldrich). After incubation, the slides were mounted with glycerol and observed under a microscope. The normal osteoblast cell lines (OSB1 and OSB2) were isolated from healthy dogs using the procedure described above (De Maria et al., 2009). To confirm their normal osteoblastic origin, part of the tissue was analysed histologically to exclude any pathological processes affecting the bone and any malignant origin (Table 3). After cellular isolation, normal phenotype of OSB1 and OSB2 was confirmed by cytological features and by the lack of the expression of Ki67, p53 and PCNA evaluated by immunocytochemistry (data not shown).

Western blot analysis

Western blot (WB) analysis was carried out on samples from the primary OSA cell lines and from a normal osteoblastic cell line (OSB). Total protein was obtained with boiling lysis buffer containing 1% SDS and 0.1 M Tris–HCl (pH 6.8). Total protein from each sample (20 μg) was separated on an 8% SDS–polyacrylamide (PAGE) gel, transferred onto a Hybond-C Extra membrane (Amersham Biosciences) and

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Source</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-A</td>
<td>Mouse monoclonal</td>
<td>1:1000</td>
</tr>
<tr>
<td>PDGF-B</td>
<td>Rabbit polyclonal</td>
<td>1:1000</td>
</tr>
<tr>
<td>PDGFRα</td>
<td>Rabbit polyclonal</td>
<td>1:1000</td>
</tr>
<tr>
<td>PDGFRβ</td>
<td>Rabbit polyclonal</td>
<td>1:1000</td>
</tr>
<tr>
<td>p-AKT</td>
<td>Rabbit polyclonal</td>
<td>1:1000</td>
</tr>
<tr>
<td>p-MAPK</td>
<td>Rabbit polyclonal</td>
<td>1:1000</td>
</tr>
<tr>
<td>α-Tubulin</td>
<td>Rabbit polyclonal</td>
<td>1:1000</td>
</tr>
</tbody>
</table>

According to the WHO guidelines (Slayter et al., 1994), while the histological grade was determined according to the system proposed by Loukopoulos and Robinson (2007).

Immunohistochemical analysis

Immunohistochemical (IHC) analysis was carried out on 4-μm paraffin sections. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 30 min at room temperature. Sections underwent high-temperature antigen unmasking by incubation with 98 °C citric acid buffer (pH 6). The anti-human primary antibodies and the dilutions used are listed in Table 1. Antibodies were detected using the avidin–biotin peroxidase complex technique with the Vectastain Elite ABC Kit (Vector Laboratories). The following positive controls were used: canine lung tissue for PDGF-A and PDGF-B, canine skin for PDGFRα and canine prostatic carcinoma for PDGFRβ. Endothelial cells were used as an internal positive control. For negative controls, the sections were incubated without the primary antibodies.

Immunolabelled slides were randomised and masked for blind examination, which was performed independently by two observers (L.M., S.I.). When there was disagreement (<5% of the slides), a consensus between the two observers was reached using a multthead microscope. Cytoplasmic immunolabelling was evaluated in neoplastic cells and in stromal cells (fibroconnective tissue within and surrounding the tumour) separately using the scoring system adopted by Donnem et al. (2008).
incubated overnight at 4 °C with the primary antibodies listed in Table 1. After incubating with a horseradish peroxidase (HRP)-linked secondary antibody diluted 1:2000 in PBS-Tween, membranes were washed in PBS-Tween for 30 min and incubated with an enhanced chemiluminescence reagent (Super Signal West Pico Mouse IgG Detection Kit, Thermo Scientific).

**Total RNA extraction and quantitative PCR expression analysis**

Total RNA was obtained from all OSA cell lines and cDNA was synthesised from 1 μg of total RNA using the QuantiTect Reverse Transcription kit (Qiagen). To determine the relative amounts of specific PDGF-A, PDGF-B, PDGFRα and PDGFRβ transcripts, the cDNA was subjected to quantitative PCR using the IQ SYBR Green Supermix (BioRad) and the IQ 5 detection system (BioRad). Primer sequences were designed using Primer Express v. 1.5 and are listed in Table 2. The level of gene expression was calculated using a relative quantification assay corresponding to the comparative cycle threshold (ΔCt) method: the amount of target, normalised to the endogenous housekeeping gene (GAPDH) and relative to the calibrator (control sample), was then transformed by 2-ΔΔCt (fold increase), where ΔΔCt = ΔCt(sample) – ΔCt(control) and ΔCt is the Ct of the target gene subtracted from the Ct of the housekeeping gene.

**PDGFR inhibition in vitro**

The ‘Penny’ cell line was treated with the specific PDGFR inhibitor AG1296 (Calbiochem) at 1 μM, 2.5 μM or 5 μM for 12, 24 or 48 h. Cytotoxicity was evaluated using the MTT method (Dacosta et al., 1994). Flow cytometric analysis of cell cycle and apoptotic activity were carried out after propidium iodide (PI) and PI versus Annexin V-FITC staining, respectively. All experiments were performed in triplicate. WB analysis of p-AKT was performed on cellular lysates obtained from Penny cells treated at different times and concentrations.

**Statistical analysis**

IHC results were grouped into contingency tables and analysed using Fisher’s exact test. The analysis of OS and DFI was performed using the Kaplan Meier method with a log rank test. P ≤ 0.05 was considered statistically significant. Data were analysed with GraphPad Prism Software v. 4.0.

**Results**

**Epidemiologic and clinical data**

Data were collected from 33 cases of appendicular OSA removed from dogs with a mean age of 7.17 ± 2.62 years (range, 2–12 years), which were predominantly males (60.6%). Eight dogs (24.24%) were mixed breed, and 25 (75.76%) were pure breed. The pure breed dogs included 7 Boxers, 3 Rottweilers, 3 Great Danes, 2 St. Bernards, 2 Newfoundlands and 1 each of the following: Beauceron, Siberian Husky, Maremma Shepherd, Malinois, German Shepherd, Greyhound, Doberman and Golden Retriever. The mean DFI observed was 311.85 ± 263.01 days (median, 251), and the mean OS was 359.06 ± 392.22 days (median, 261).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Grade</th>
<th>DFI (days)</th>
<th>OS (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall</td>
<td>0.5</td>
<td>m</td>
<td>Osteoblastic productive osteosarcoma</td>
<td>III</td>
<td>No relapse</td>
<td>Still alive</td>
</tr>
<tr>
<td>Penny</td>
<td>8</td>
<td>f</td>
<td>Osteoblastic productive osteosarcoma</td>
<td>III</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Dark</td>
<td>10</td>
<td>m</td>
<td>Lymphonodal metastasis of osteoblastic productive osteosarcoma</td>
<td>III</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Sky</td>
<td>9</td>
<td>m</td>
<td>Osteoblastic productive osteosarcoma</td>
<td>III</td>
<td>83</td>
<td>98</td>
</tr>
<tr>
<td>Pedro</td>
<td>9</td>
<td>m</td>
<td>Chondroblastic osteosarcoma</td>
<td>III</td>
<td>188</td>
<td>Still alive</td>
</tr>
<tr>
<td>Lord</td>
<td>8</td>
<td>m</td>
<td>Osteoblastic productive osteosarcoma</td>
<td>II</td>
<td>No relapse</td>
<td>Still alive</td>
</tr>
<tr>
<td>Desmond</td>
<td>4</td>
<td>m</td>
<td>Osteoblastic productive osteosarcoma</td>
<td>III</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>OSB1</td>
<td>8</td>
<td>f</td>
<td>Normal bone</td>
<td>III</td>
<td>/</td>
<td>ND</td>
</tr>
<tr>
<td>OSB2</td>
<td>5</td>
<td>m</td>
<td>Normal bone</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

DFI, disease free interval; OS, overall survival; ND, not determined.

**Histopathology**

The following distribution was observed: 19 cases (57.58%) of osteoblastic productive OSA, seven cases of (21.21%) chondroblastic OSA, four cases (12.12%) of fibroblastic OSA, two cases (6.06%) of unproductive osteoblastic OSA and one case (3.03%) of giant cell OSA. The cases were categorized into 8 (24.24%) grade I, 13 (39.40%) grade II and 12 (36.36%) grade III OSAs. Cell lines were collected from seven OSA and from one lymph nodal metastasis (Dark). Histopathological examination was used to diagnose and grade the samples as listed in Table 3.

**Immunohistochemistry**

The results of IHC staining for PDGF-A, PDGF-B, PDGFRα and PDGFRβ in OSAs of different grades are summarised in Table 4. PDGFRα and PDGFR-B immunolabelling was observed in the cytoplasm mainly at the tumour border. In particular, PDGF A was detected in 42.42% (14/33) of cases in neoplastic cells (Fig. 1) and in 48.48% (16/33) of cases in stromal cells (Fig. 2), while PDGF B was observed in 60.61% (20/33) of cases in neoplastic cells (Fig. 3) and in 18.18% (6/33) of cases in stromal cells.

Staining for PDGFRα and PDGFRβ was observed with a uniform distribution within the neoplastic tissue, mainly in the cytoplasm and in some cases with a brighter membrane labelling (Appendix A, Supplementary file). PDGFRα immunostaining was observed in 78.79% (26/33) of cases in neoplastic cells (Fig. 4) and in 21.21% of cases (7/33) in stromal cells. PDGFRβ was observed in neoplastic cells in 81.81% (27/33) of cases and in stromal cells in 24.24% (8/33) of cases (Fig. 5). Immunolabelling of PDGFs and PDGFRs was also detected in the cytoplasm of endothelial cells of normal capillaries and blood vessels.

Statistical analyses comparing the protein expression of PDGF-A, PDGF-B, PDGFRα and PDGFRβ and their locations (tumoural or stromal) as well as histological diagnosis and grading showed no statistical associations. Long rank tests performed to compare the
IHC results and the OS and DFI times showed no statistical correlation between PDGFs and PDGFRs staining and survival.

Culture of the primary osteosarcoma cell lines

Six primary OSA cell lines, one metastatic OSA cell line and two normal primary osteoblastic cell lines were characterised (Table 3). The morphological findings and alkaline phosphatase activity were assessed to confirm the osteoblastic origins of the cultured cells (Appendix A, Supplementary file).

Molecular investigations

WB analyses of canine PDGFRα and β, PDGF-A, total AKT, p-AKT and p-MAPK were performed. Specific bands corresponding to canine PDGFR α (195 kDa) and β (195 kDa) and to PDGF-A (31 kDa) were found in all OSA cell lines except for the Lord cell line, which did not express PDGFRα (Fig. 6). Higher expression of PDGFRα was observed in the Wall and Penny cell lines, while PDGFRβ was detected at a very low level in all of the OSA cell lines.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>PDGF-A</th>
<th>PDGF-B</th>
<th>PDGFRα</th>
<th>PDGFRβ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumour</td>
<td>Stroma</td>
<td>Tumour</td>
<td>Stroma</td>
</tr>
<tr>
<td>I grade</td>
<td>4/8 (50.00%)</td>
<td>6/8 (75.00%)</td>
<td>5/8 (62.50%)</td>
<td>2/8 (25.00%)</td>
</tr>
<tr>
<td>II grade</td>
<td>4/13 (30.77%)</td>
<td>6/13 (46.15%)</td>
<td>7/13 (53.85%)</td>
<td>2/13 (15.38%)</td>
</tr>
<tr>
<td>III grade</td>
<td>6/12 (50.00%)</td>
<td>4/12 (33.33%)</td>
<td>8/12 (66.67%)</td>
<td>2/12 (16.67%)</td>
</tr>
<tr>
<td>Total OSA</td>
<td>14/33 (42.42%)</td>
<td>16/33 (48.48%)</td>
<td>20/33 (60.61%)</td>
<td>6/33 (18.18%)</td>
</tr>
</tbody>
</table>

+/tot, positive cases/total cases.

Fig. 1. Osteoblastic productive osteosarcoma. Neoplastic cells are characterised by diffuse and strong cytoplasmic immunolabelling for PDGF-A. Streptavidin–biotin–peroxidase method. Mayer’s haematoxylin counterstain.

Fig. 2. Chondroblastic osteosarcoma. Stromal cells characterised by strong cytoplasmic immunolabelling for PDGF-A and neoplastic cells PDGF-A negative. Streptavidin–biotin–peroxidase method. Mayer’s haematoxylin counterstain.

Fig. 3. Osteoblastic productive osteosarcoma. Neoplastic cells are characterised by diffuse and weak cytoplasmic immunolabelling for PDGFRα. Streptavidin–biotin–peroxidase method. Mayer’s haematoxylin counterstain.

Fig. 4. Chondroblastic osteosarcoma. Neoplastic cells are characterised by diffuse and strong cytoplasmic positivity for PDGFRα. Streptavidin–biotin–peroxidase method. Mayer’s haematoxylin counterstain.
Discussion

OSA is the most frequently diagnosed cancer in the bone of dogs. Several authors have demonstrated that this tumour shows many similarities with human OSA (De Maria et al., 2009; Morello et al., 2011), which is one of the most frequent tumours of childhood and adolescence (Kempf-Biellack et al., 2005; Ottaviani and Jaffe, 2009). Despite the use of aggressive treatments such as adjuvant chemotherapy and wide tumour resection, OSA remains a major cause of fatality in both species; therefore, new therapeutic strategies based on targeted therapy may improve the treatment and prognosis of OSA.

In the current study, we demonstrated that PDGFRs and their specific ligands, PDGF-A and -B, are overexpressed in canine OSA and that they may represent suitable targets for specific (targeted) therapy. Because PDGFs and PDGFRs are physiologically expressed in a variety of cell types, such as fibroblasts, vascular smooth muscle cells and endothelial cells (Alvarez et al., 2006), immunolabelling of PDGs and PDGFRs was detected not only in tumour tissue but also in the stromal compartment (fibroblasts and endothelial cells). PDGFRα and PDGFRβ were expressed in 78.79% (26/33) and 81.22% (27/33) of tumour cells, respectively, while expression in the stromal compartment was 21.21% and 24.24%, respectively. These data are similar to those previously reported for humans (Kubo et al., 2008), where PDGFRα and PDGFRβ were present in 79.8% and in 86% of tumours, respectively.

A difference in PDGF-A expression was observed between canine (42%) and the reported value for human (80.4%) samples. In contrast, PDGF-B is expressed in 60.61% of canine OSAs and in 75.4% of human OSAs. No significant correlation was observed between the expression of these molecules and survival, histological grading or age. These results are different from data in humans, since one study has demonstrated that expression of PDGFRα and PDGF-A are correlated with inferior event-free survival (Kubo et al., 2008) and another study showed that PDGF-A expression was associated with tumour progression (Sulzbacher et al., 2003). The co-expression of PDGFRs and their specific ligands in our samples suggests a functional autocrine and/or paracrine stimulation loop that promotes both tumour growth and angiogenesis, as suggested by Östman (2004).

We found that 24% of OSA expressed PDGFRβ in the stromal compartment. This is in accordance with humans, in which a series of IHC studies have shown prominent PDGFRβ expression in the stroma of several solid tumours (Sundberg et al., 1993; Pontén et al., 1994; Fjällskog et al., 2003); however, a significant correlation between PDGFRα and PDGF-A and B has not been previously reported. On the basis of the IHC results, we can determine that PDGF and PDGF expression does not have a prognostic value in canine OSA, but based on their high expression, they may represent important therapeutic targets.

To better understand the role of PDGFRs and PDGFs in canine OSA, seven primary cell lines were characterised, and the molecular pathways associated with PDGFR investigated. Six cell lines overexpressed PDGFRα compared to normal OSBs, with the exception of the Lord cell line. PDGFRβ and PDGF-A were expressed in all cell lines including OSB, the normal cell line. PDG-F-A expression is consistent with what has been demonstrated in humans, where a
low level of PDGF-A is physiologically expressed in osteoblasts during osteogenesis (Horner et al., 1996), while overexpression is observed in OSA (Sulzbacher et al., 2000, 2003).

All cell lines expressing PDGFRs show activation of p-AKT, and five cell lines showed activation of p-MAPK. These results confirm the data available in the human literature, in which the major signal transduction pathways activated by PDGFRα and PDGFRβ are the PI3K (phosphatidylinositol 3-kinase), Ras mitogen-activated protein kinase and phospholipase Cγ pathways (Liu et al., 2011). On the basis of these results, we investigated the inhibitory effect of AG1296 on AKT phosphorylation in the Penny cell line, which overexpresses PDGFRs and has a high level of p-AKT. We demonstrated that AG1296 can decrease p-AKT in a dose- and time-dependent manner (Fig. 8). These data are similar to the effects observed in human OSA cell lines, where imatinib mesylate (ST1571) in vitro decreased p-AKT and p-MAPK levels (Kubo et al., 2008). However, AG1296 did not induce apoptosis or inhibit proliferation. Apoptosis and proliferation are not solely p-AKT-dependent and other cellular pathways that sustain tumour growth and anti-apoptotic pathways exist, including MET (De Maria et al., 2009), the IGF-1 and IGF1R pathways (MacEwen et al., 2004) or the STAT3 pathway (Fossey et al., 2009).

Conclusions

Both PDGFRs and PDGFs are probably involved in the pathogenesis of canine OSA and their co-expression suggests a functional autocrine and/or paracrine loop of growth stimulation. Molecular studies performed on primary OSA cell lines confirmed that the downstream effectors of PDGFRs are p-AKT- and MAPK-like in humans. Our data further confirm that canine OSA shows similarity to the human disease not only in clinical and histological findings, but also at the molecular level.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could appropriately influence or bias the content of the paper.

Acknowledgements

This work was funded by the Italian Ministry of University and Scientific Research (PRIN 2008). The authors wish to thank Alessandra Sereno for technical support and the Reference Centre of Comparative Pathology Bruno Maria Zaini of the Faculty of Veterinary Medicine of Turin.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tvjl.2012.05.003.