Histol Histopathol (2012) 27: 601-607
DOI: 10.14670/HH-27.601

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Expression of platelet-derived growth factor and its receptors in spontaneous canine hemangiosarcoma and cutaneous hemangioma

S. Abou Asa[1,4], A. Murai[1], M. Murakami[1,3], Y. Hoshino[2,3], T. Mori[2,3], K. Maruo[2,3], A. Khater[4], A. El-sawak[4], E. Abd el-Aziz[4], T. Yanai[1] and H. Sakai[1,3]

[1]Laboratory of Veterinary Pathology, [2]Clinical Oncology, Department of Veterinary Medicine, [3]Comparative Cancer Center, Faculty of Applied Biological Sciences, Gifu University and [4]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Kafr El-Sheikh, Egypt

Summary. Hemangiosarcoma (HSA) is a malignant neoplasia of vascular endothelial cells (ECs). Our previous report on the expression of vascular endothelial growth factor, basic fibroblast growth factor, and their receptors in canine HSA suggested an autocrine/paracrine mechanism of tumor growth. However, the influence of other angiogenic growth factors in canine HSA was not elucidated; therefore, the expression of platelet-derived growth factor (PDGF) and its receptors was investigated by immunohistochemical analysis. Forty-six canine HSAs and 21 canine cutaneous hemangiomas (HAs) were analyzed. For immunohistochemistry, anti-PDGF-BB, anti-PDGFR-α, and anti-PDGFR-ß antibodies were utilized as primary antibodies. Immunoreactivities were scored as strongly positive (>25% positive neoplastic cells), weakly positive (1-25% positive neoplastic cells), and negative if not staining at all. In cutaneous HA, 33.3% and 57.1% of cases were strongly and weakly positive, respectively, and 43.5% and 13.0% of HSAs were strongly and weakly positive for PDGF-BB, respectively. Moreover, 38.1% and 28.6% of cutaneous HAs cases were strongly and weakly positive, respectively, and 23.9% and 4.3% of HSAs cases were strongly and weakly positive, respectively, for PDGFR-α. Thirty-five HSAs cases (76.1%) were strongly positive, and the remaining 11 (23.9%) were weakly positive for PDGFR-ß. In contrast, 18 (72.0%) cutaneous HAs were negative, and only 3 cases (12.0%) were weakly positive, for PDGFR-ß. The proportion of strongly positive cases of HSAs was significantly higher than that of cutaneous HA for PDGFR-ß ($P<0.01$), while PDGFR-α was highly expressed in cutaneous HA and may be related to pathogenesis of cutaneous HA. Therefore, PDGFR-ß may be associated with the malignant nature of canine HSA.

Key words: Dog, Hemangiosarcoma, Immunohistochemistry, PDGF-BB, PDGF receptors

Offprint requests to: Hiroki Sakai, Laboratory of Veterinary Pathology, Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan. e-mail: shiroki@gifu-u.ac.jp

Introduction

Hemangiosarcoma (HSA) is a rare malignant mesenchymal vasoformative neoplasia, which arises in various soft tissues and visceral organs, accounting for less than 1% of all sarcomas in humans (Mobini, 2009; Lucas, 2009). It is an aggressive high-grade sarcoma with about 15% of patients presenting metastatic disease at the time of diagnosis (Fury et al., 2005; Fayette et al., 2007), and a 5-year survival rate of 31-43% with non-metastatic HSA (Mark et al., 1996; Fury et al., 2005; Fayette et al., 2007). HSA occurs more commonly in dogs than in any other species, including humans, and the incidence of canine HSA is reported to be 12-21% of all mesenchymal neoplasms (Thamm, 2007). As in human HSA, local invasion and systemic metastasis of canine HSA are common (Oksanen, 1978; Brown et al., 1985), and the median survival time of dogs diagnosed with HSA is a little more than 6 months (Hammer et al., 1991; Clifford et al., 2000). Therefore, with regard to comparative oncology, spontaneous canine visceral or cutaneous HSA is an appropriate model for the investigation of spontaneous human HSA (Fosmire et al., 2004; Kodama et al., 2009; Tamburini et al., 2009).

Angiogenesis plays a crucial role in many pathophysiological conditions. Blood vessel formation is

a multi-step process regulated by proangiogenic and antiangiogenic factors (Folkman, 1971). Vascular endothelial growth factor (VEGF) is the most investigated and most efficient angiogenic molecule (Ferrara and Henzel, 1989), strongly stimulating proliferation and migration of endothelial cells(ECs)in both normal and pathophysiological conditions (Ferrara, 1999, 2001). In addition to VEGF, other growth factors, such as basic fibroblast growth factor (bFGF), transforming growth factor, hepatocyte growth factor, angiopoietin-1, and platelet-derived growth factor (PDGF) have a significant proangiogenic effect (Ferrara and Kerbel, 2005). In human and canine HSA, the expression of VEGF, bFGF, and their receptors in neoplastic cells was investigated, which suggested that these factors influence not only angiogenesis but also proliferation of neoplastic ECs (Hashimoto et al., 1995; Yonemaru et al., 2006; Tokuyama et al., 2010).

Only in recent decades have PDGFs, synthesized by several different cell types, been extensively investigated in normal and tumor-associated angiogenesis. Specific PDGF receptors were shown to be expressed by a large variety of normal and neoplastic cells, as validated by the effectiveness of specific inhibitors already in use as therapeutic drugs in some human tumors (Raica and Cimpean, 2010). PDGF signals through 2 receptor tyrosine kinases, PDGFR-α and PDGFR-ß, which stimulate various cellular functions, such as differentiation, proliferation, wound healing, and angiogenesis (Heldin and Westermark, 1999). Studies, utilizing gene targeting in mice, on PDGF and PDGFRs have shown that PDGF plays an important role in paracrine growth in vascular development (Magnusson et al., 2007). Moreover, defective synthesis of PDGF, and associated autocrine growth stimulation, may be an important step in neoplastic transformation of PDGFR-positive cells. To our knowledge, there are no reports on the expression of PDGF and PDGFRs in canine HSA and cutaneous hemangioma (HA). Therefore, in this study, we investigated the expression of PDGF-BB, PDGFR-α, and PDGFR-ß in canine HSA and cutaneous HA by immunohistochemical analysis.

Materials and methods

Samples

Archival surgically removed samples from the Department of Veterinary Pathology, Gifu University, were used for this study. All tumors were considered as primary tumors by clinical examination and surgical findings. There were 21 samples of canine cutaneous HA from 12 males (6-14 years) and 9 females (7-13 years), which comprised Golden Retriever (5 cases), followed by Labrador Retriever (3 cases), then other breeds, such as mixed breeds and Shih Tzus. All cases of cutaneous HA were of the cavernous subtype, and displayed large, regular and well defined vascular spaces filled with erythrocytes and completely lined by a single layer of endothelial cells overlying thin collagenous septa. There were 46 samples of canine HSA (36 from the spleen, 5 from the subcutis, 2 from the liver, 1 from the nictitating membrane, 1 from the kidney, and 1 from an abdominal mass- site of origin was unknown ,but it was attached to the mesentery and did not extend to the spleen or liver).The HSA samples were taken from25 males(8-13 years), 20 females (8-15 years) and 1 dog of unknown age and sex. HSA also tended to occur more frequently in Golden Retriever (14 cases), followed by mixed breeds (8 cases), Beagle (5 cases), Labrador Retriever (4 cases),and other breeds(15 cases). The samples had been surgically removed and immediately fixed in 10 % neutral buffered formalin, then embedded in paraffin wax, and sectioned for either hematoxylin and eosin(H&E) or immunohistochemcal staining. The diagnosis for each tumor had been previously confirmed by examination of H&E -stained slides. In HSA, neoplastic cells were spindle or polygonal to ovoid shaped, had prominent pleomorphic, hyperchromatic nuclei showing frequent mitotic figures and formed irregular vascular clefts or channels. According to the classification by Bertazzolo et al. (2005), HSA samples were divided into poorly differentiated type (12 cases), where the neoplastic cells formed solid tumors, and well differentiated type (34 cases) where well- defined vascular clefts were lined by the neoplastic cells. In the peritumoral tissue, particularly in the splenic HSAs, there were areas of hemorrhage, which occurred diffusely through the parenchyma in some cases and as small patches evidenced by the presence of hematoidin pigment in others. All HAs and HSAs were confirmed by the expression of von Willebrand factor and CD31 by immunolabeling with specific antibodies (von Willebrand factor rabbit antibody and CD31 mouse monoclonal antibody; Dako, Glostrup, Denmark).

Immunohistochemical analysis

Immunohistochemical staining was performed using 4-μm thick paraffin-embedded sections. The sections were deparaffinized in xylene and rehydrated in graded ethanol. For antigen retrieval, the sections were immersed in Target Retrieval Solution High pH (Dako) and autoclaved at 105°C, for 10 min for PDGF-BB, and for 20 min for PDGFR-α and PDGFR-ß. Endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 in methanol for 20 min at room temperature (RT). To prevent the binding of nonspecific proteins, the sections were treated with Protein Block Serum Free (Dako) for 30 min at RT. Immunolabeling of PDGF-BB, PDGFR-α, and PDGFR-ß was performed on all samples, using anti-PDGF-BB (rabbit polyclonal, ab23914; Abcam, Cambridge, UK), anti-PDGFR-ß (rabbit polyclonal; ThermoFischer Scientific, Waltham, USA), and anti-PDGFR-ß (rabbit monoclonal antibody28E1; Cell Signaling Technology, Danvers, USA) antibodies. Sections were incubated with primary antibodies (PDGF-BB [1:1000], PDGFR-α [1:500], and PDGFR-ß

[1:50]) overnight at 4°C. Next, the sections were washed with phosphate-buffered saline (PBS) and incubated with the appropriate secondary antibody (EnVision + System, HRP labeled polymer anti-rabbit, Dako) for 30 min at RT. After washing the sections with PBS, 3,3'-diaminobenzidinetetrahydrochloride (Liquid DAB + Substrate Chromogen System, Dako) was applied to display binding. The sections were then washed in distilled water and counterstained with Mayer's hematoxylin prior to examination.

Staining of inflammatory cells (such as macrophages), vascular smooth muscle cells, and fibroblasts was used as the internal control for immunoreactivity (Dalla-Favera et al., 1982; Vassbotn et al., 1994; Nakagawa et al., 2010).

Scoring of immunohistochemical results

The percentage of neoplastic cells with positive cytoplasmic labeling of PDGF-BB, PDGFR-α, and PDGFR-ß was determined by analyzing 1000 cells (obtained from 10 x 400 high power fields). Following the classification by Kubo et al. (2008), the cases were divided into 3 categories based on the percentage of neoplastic cells showing positive labeling: negative (no positive cells), weakly positive (1-25% positive cells), and strongly positive (>25% positive cells).

A chi-square test was used to compare the proportion of positive cases of PDGF-BB, PDGFR-α, and PDGFR-ß between HSA and cutaneous HA, and $P<0.05$ was considered significant.

Results

Immunoreactivities for PDGF-BB, PDGFR-α, and PDGFR-ß were observed in the cytoplasm of the neoplastic cells (Fig. 1G). Macrophages, vascular smooth muscle cells, and fibroblasts were used as internal controls and showed varying degrees of positive cytoplasmic staining (Fig. 1F,G). Seven cases (33.3%) and 12 cases (57.1%) of cutaneous HA were strongly and weakly positive, respectively, for PDGF-BB, and 20 cases (43.5%) and 6 cases (13.0%) of HSA were strongly and weakly positive for PDGF-BB, respectively. Eight cases (38.1%) and 6 cases (28.6%) of cutaneous HA were strongly (Fig.1D) and weakly positive, respectively, for PDGFR-α, and 11 cases (23.9%) and 2 cases (4.3%) of HSA were strongly and weakly positive for PDGFR-α (Fig. 1C), respectively. Immunoreactivity for PDGFR-ß differed between HSA and cutaneous HA. Thirty-five HSAs were strongly positive for PDGFR-ß (Fig. 1A,B), and the remaining 11 were weakly positive. In contrast, only 3 cases of cutaneous HA were weakly positive for PDGFR-ß (Fig. 1E), and the remaining 18 were negative. The proportion of cases of HSA showing strong positive immunoreactivity for PDGFR-ß was significantly higher than that of cutaneous HA ($P<0.01$). There was no other notable immunoreactivity in the normal splenic parenchyma in the HSA cases, and the skin in cutaneous HA cases. The data are summarized graphically in Fig. 2.

Discussion

Hemangiosarcoma which is rare in humans, highly aggressive and painless, and clinical signs are usually not evident until advanced stages when the tumors are resistant to most treatments (Mark et al., 1996; Fury et al., 2005; Fayette et al., 2007). Owing to the availability of samples, canine heamangiosarcoma is used as a model for studying the molecular pathogenesis of human HSA and defining a possible treatment target in humans and dogs, and may also assist in the development of an early diagnostic method (Fosmire et al., 2004; Yonemaru et al., 2006; Kodama et al., 2009; Tamburini et al., 2009). Some important factors associated with the malignant nature of canine HSA have been previously reported; the expression of VEGF-A, bFGF, VEGFRs, FGFR, CD117, and CD44 in HSAs suggested that these molecules might be potential targets for molecular interventional therapy for this tumor (Yonemaru et al., 2006; Sabattini and Bettini, 2009). It was also suggested that mast cells may play a role in the pathogenesis of cutaneous HA (Sabattini and Bettini 2009).

In the present study, all cases of HSA were positive for PDGFR-ß, and the proportion of strongly positive HSA cases was significantly higher than that of cutaneous HA ($P<0.01$). Only 14.3% of cutaneous HA cases were weakly positive for PDGFR-ß. In addition, the proportion of PDGF-BB positive cases of HSA tended to be higher than that of cutaneous HA, although there was no significant difference.

Recently, PDGFs and PDGFRs have been shown to play a role in certain human malignant tumors, such as gastrointestinal stromal tumor, dermatofibrosarcoma (McCarthy et al., 2010), and glioblastomas (Lokker et al., 2002). PDGF signaling was also implicated in choroid plexus tumors as phosphorylated PDGFR-ß was found to be significantly higher than phosphorylated PDGFR-α and in immortalized choroid plexus epithelial cell lines, PDGFR-ß expression was significantly attenuated by imatinib (Koos et al., 2009). Moreover, the expression level of PDGFR-ß was significantly higher in esophageal carcinoma tumor tissues than in normal tissues (Zhang et al., 2006), and PDGF signaling influenced PDGF-BB -induced c-Jun expression and promoted the growth of a human esophageal carcinoma cell line by providing a growth advantage and preventing apoptosis, suggesting autocrine PDGF stimulation of esophageal carcinoma cells (Liu et al., 1996).

Although there are few reports on PDGFs and their receptors in animal tumors, in type 2 bovine papilloma virus-induced bovine urothelial carcinoma, the viral E5 oncoprotein, which bound to and activated the PDGFR-ß in vitro (Petti et al., 1991; DiMaio et al., 2000), and phosphorylated PDGFR-ß formed a stable complex in the carcinoma cells, thereby inducing dimerization and

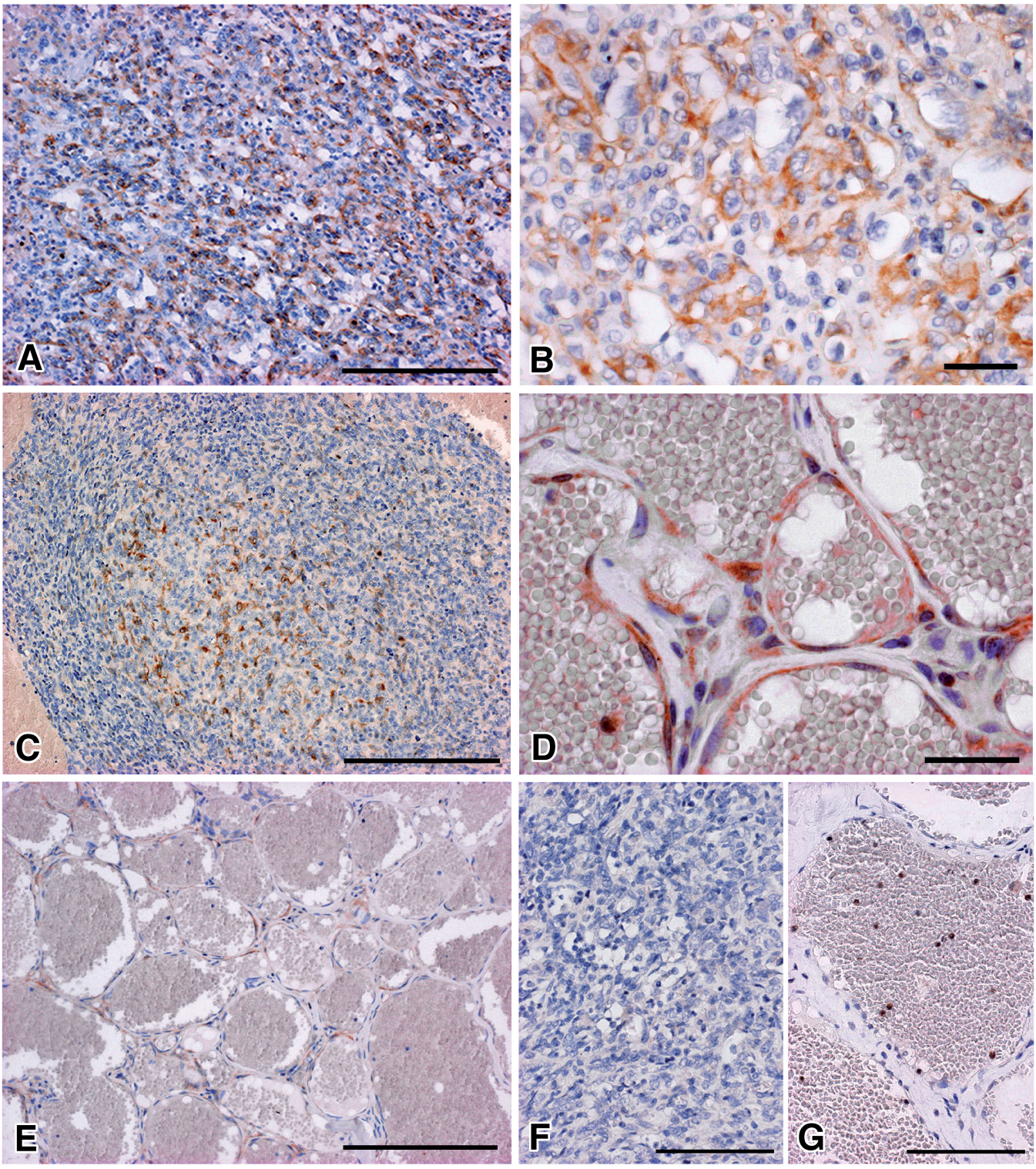

Fig. 1. Immunohistochemistry of PDGF-BB and PDGFRs in canine HSA and HA. **A.** Spleen, HSA, showing strong positive immunoreactivity for PDGFR-ß. Positive neoplastic cells are distributed throughout the tumor tissue. **B.** Spleen, HSA, showing High magnification of strong positive immunoreactivity for PDGFR-ß in. Positive reaction is observed in the cytoplasm of neoplastic cells. **C.** Spleen, HSA, showing weak positive immunoreactivity for PDGFR-ß. The cytoplasm of some neoplastic cells are positive and usually this cells are distributed around the major blood vessels. **D.** Skin, HA, showing strongly positive immunoreactivity for PDGFR-α inform of Small, flattened positive neoplastic endothelial cells. Some interstitial cells and inflammatory cells, which may be macrophages, are positive. **E.** Skin, HA, showing weakly positive immunoreactivity for PDGFR-ß. Positive ECs are scattered throughout the field. **F and G.** Negative control for PDGFR-BB. **F** is negative spleen for HSA and **G** is negative skin for HA. Inflammatory cells are positive. Bars: A, C, E, 200 μm; B, D, 50 μm; F, G, 100 μm.

transphosphorylation of tyrosine residues in the cytoplasmic domain of the receptor itself (Borzacchiello et al., 2006). In canine sarcoma, Levine (2002) reported that all the investigated canine osteosarcoma cell lines expressed PDGFR-α and 1 cell line(designated CO8) expressed higher sis mRNA-encoded PDGF-B. The previous study also discussed the importance of PDGF in promoting cell proliferation, migration, and cell survival, and that sis activation potentially contributed to the pathogenesis of a subset of canine osteosarcoma. Thus, the activation of PDGFRs plays an important role in carcinogenesis of some neoplasms in humans and animals. Besides a high PDGF-BB concentration, the overexpression of PDGFRs may exacerbate the effect of PDGF-BB, which supports the hypothesis that the PDGF-BB/PDGFR-ß pathway initiates the autocrine/paracrine mechanism responsible for the proliferation of HSA, which is similar to carcinogenesis in the canine osteosarcoma cell lines. We could not identify the cause of receptor overexpression in the present study; therefore, it warrants further investigation. The present results suggest that overexpression of PDGFR-ß may be associated with the malignant nature of canine HSA compared with cutaneous HA.

In contrast to PDGFR-ß, PDGFR-α tended to be more strongly expressed in cutaneous HA than in HSA. Overexpression of PDGFR proteins was reported in human osteosarcoma (Kubo et al., 2008) through coexpression of PDGF-A and PDGFR-α, but not PDGF-B and PDGFR-ß, and this was associated with a worse prognosis in human osteosarcoma. However, some authors reported a direct antiangiogenic effect of the PDGF-A/PDGFR-α pathway by inhibition of the angiogenic properties of bFGF; this is in contrast to the PDGF-BB/PDGFR-ß, which is proangiogenic (De Marchis et al., 2002).The function of the PDGF-A/PDGFR-α pathway may vary in different cell types. In canine cutaneous HA, immunoreactivities for bFGF of neoplastic ECs were weak (Yonemaru et al., 2006). In the present study, we only investigated cutaneous HA, therefore, we were unable to conclude whether the result was specific to cutaneous HA or not. However, in addition to the low expression of bFGF in the tumor microenvironment, it is possible to antagonize the effect of bFGF by activation of the PDGF-A/PDGFR-α pathway and maintain tumor cells of canine HA in the quiescent condition.

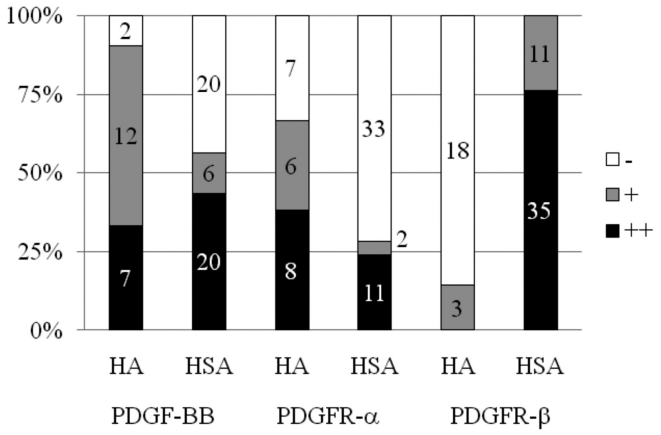

Fig. 2. Summary of immunohistochemical results for PDGF-BB, PDGFR-α and PDGFR-ß. The white, gray, and black columns indicate negative (-), weakly positive (+), and strongly positive (++) staining, respectively. The numbers appearing in or near the columns indicate the number of cases showing the corresponding immunoreactivity.

The spleen is known to be the most common site of canine HSA (Goldschmidt and Hendrick, 2002); therefore, splenic HSAs accounted for most of the samples in the present study. In the HSAs, there was no significant difference in the rate of positive cases between splenic HSAs and non splenic HSAs (30.5% vs. 20% for PDGFR-α and 50% vs.58% for PDGF-BB, respectively, and all HSA cases were positive for PDGFR-ß). Therefore, we considered that there were no site-specific differences in HSA, although further investigation is needed due to the very small number of non- splenic HSAs.

The levels of secreted PDGF-BB and VEGF were strongly correlated in human ovarian tumors, suggesting that the 2 pathways interconnect. Furthermore, in PDGFR- expressing immortalized ovarian cancer cells, PDGF potently induced VEGF secretion, while treatment with imatinib mesylate reduced PDGF-stimulated VEGF production to basal levels (Matei et al., 2007). Coexpression of VEGF-A and PDGF-B is necessary to induce the formation of invasive, solid squamous skin cell carcinomas with a stable, functional tumor vasculature (Lederle et al., 2010). Previously, we reported intense positive staining of VEGF-A proteins and mRNA in neoplastic ECs of canine HSA by immunohistochemical analysis and in situ hybridization, whereas they were only slightly positive or not detected in cutaneous HA (Yonemaru et al., 2006). As all HSA cases in this study were positive for PDGFR-ß, the expression of VEGF in canine HSA may be associated with the activation of PDGFR-ß. Moreover, increased PDGFR-ß activity is also associated with the overexpression of VEGFR-2, and results in increased sprouting and blood vessel formation (Magnusson et al., 2007). The canine HSA that showed intense staining for VEGFR-2 had high proliferative activity, which was reflected by a high Ki-67 labeling index (Yonemaru et al., 2006); therefore, PDGFR-ß may significantly influence VEGF-mediated autocrine/paracrine growth of canine HSA.

In conclusion, PDGF signaling has been shown to support growth in a variety of human and canine tumors and this signaling differs among tumor types, some through PDGF/PDGFR-α, or PDGF/PDGFR-ß and c-Kit and others through interconnection between other growth factors such as VEGF. Overexpression of PDGFR-ß was observed in canine HSA, and approximately half of the cases were also positive for

PDGF-BB. Therefore, the activation of the PDGFR-ß/PDGF-BB pathway may be associated with the malignant nature of canine HSA. In contrast, PDGFR-α expression was greater in cutaneous HA than in HSA; as a result, PDGFR-α may play a role in the benign nature of cutaneous HA. Notably, the PDGF-BB/PDGFR-ß pathway is a potential target for molecular therapy in canine HSA (which shows significant expression of PDGFR-ß in malignant ECs) by using, for example, inhibitors for receptor tyrosine kinase or neutralized antibodies for growth factors.

References

Bertazzolo W., Dellorco M., Benfanti U., Ghisteni G.,. Caniatti M., Masserdotti C., Antoniazzi E., Crippa L. and Roccabianca P. (2005). Canine angiosarcoma: cytologic, histologic, and immunohistochemical correlations. Vet. Clin. Pathol. 34, 28-34.

Borzacchiello G., Russo V., Gentile F., Roperto F., Venuti A., Nitsch L., Campo M.S. and Roperto S. (2006).Bovine papillomavirus E5 oncoprotein binds to the activated form of the platelet-derived growth factor b receptor in naturally occurring bovine urinary bladder tumours. Oncogene 25, 1251-1260.

Brown N.O., Patnaik A.K. and MacEwen E.G. (1985). Canine hemangiosarcoma: retrospective analysis of 104 cases. J. Am. Vet. Med. Assoc. 186, 56-58.

Clifford C.A., Mackin A.J. and Henry C. J. (2000). Treatment of canine hemangiosarcoma: and beyond. J. Vet. Intern. Med. 14, 479-485.

Dalla-Favera R., Gallo R.C., Giallongo A. and Croce C.M. (1982). Chromosomal localization of the human homolog (c-sis) of the simian sarcoma virus onc gene. Science 218, 686-688.

De Marchis F., Ribatti D., Giampietri C., Lentini A., Faraone D., Scoccianti M., Capogrossi M.C. and Facchiano A. (2002) Platelet-derived growth factor inhibits basic fibroblast growth factor angiogenic properties in vitro and in vivo through its alpha receptor. Blood 99, 2045-2053.

DiMaio D., Lai C.C. and Mattoon D. (2000). The platelet-derived growth factor beta receptor as a target of the bovine papillomavirus E5 protein. Cytokine Growth Factor. Rev. 11,283-293.

Fayette J., Martin E., Piperno-Neumann S., Le Cesne A., Robert C., Bonvalot S., Ranchère D., Pouillart P., Coindre J.M. and Blay J.Y. (2007). Angiosarcomas, a heterogeneous group of sarcomas with specific behavior depending on primary site: a retrospective study of 161 cases. Ann. Oncol. 18, 2030-2036.

Ferrara N. (1999). Molecular and biological properties of vascular endothelial growth factor. J. Mol. Med. 77, 527-543.

Ferrara N. (2001). Role of vascular endothelial growth factor in regulation of physiological angiogenesis. Am. J. Physiol. Cell. Physiol. 280, 1358-1366.

Ferrara N. and Henzel W.J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem. Biophys. Res. Commun. 161, 851-858.

Ferrara N. and Kerbel K.S. (2005). Angiogenesis as a therapeutic target. Nature Insight 438, 967-974.

Folkman J. (1971). Tumor angiogenesis: therapeutic implications. New Engl. J. Med. 285, 1182-1186.

Fosmire S.P., Dickerson E.B., Scott A.M., Bianco S.R., Pettengill M.J., Meylemans H., Padilla M., Frazer-Abel A.A., Akhtar N., Getzy D.M., Wojcieszyn J., Breen M., Helfand S.C. and Modiano J.F. (2004). Canine malignant hemangiosarcoma as a model of primitive angiogenic endothelium. Lab. Invest. 84, 562-572.

Fury M.G., Antonescu C.R., Van Zee K.J., Brennan M.F. and Maki R.G. (2005). A 14-year retrospective review of angiosarcoma: clinical characteristics, prognostic factors, and treatment outcomes with surgery and chemotherapy. Cancer J. 11, 241-247.

Goldschmidt M.H. and Hendrick M.J. (2002). Tumors of the skin and soft tissue. In: Tumors in domestic animals. 4th ed, Meuten D.J. (ed). pp 45-119.

Hammer A.S., Couto C.G.., Filppi J., Getzy D. and Shank K. (1991). Efficacy and toxicity of VAC chemotherapy (vincristine, doxorubicin, and cyclophosphamide) in dogs with hemangiosarcoma. J. Vet. Intern. Med. 5, 160-166.

Hashimoto M., Ohsawa M., Ohnishi A., Naka N., Hirota S., Kitamura Y. and Aozasa K. (1995). Expression of vascular endothelial growth factor and its receptor mRNA in angiosarcoma. Lab. Invest. 73, 859-863.

Heldin C.H. and Westermark B. (1999). Mechanism of action and in vivo role of platelet-derived growth factor. Physiol. Rev. 79, 1283-1300.

Kodama A., Sakai H., Matsuura S., Murakami M., Murai A., Mori T., Maruo K., Kimura T., Masegi T. and Yanai T. (2009). Establishment of canine hemangiosarcoma xenograft models expressing endothelial growth factors, their receptors, and angiogenesis-associated homeobox genes. BMC Cancer 14, 9-363.

Koos B., Paulsson J., Jarvius M., Sanchez B.C., Wrede B., Mertsch S., Jeibmann A., Kruse A., Peters O., Wolff J.E., Galla H.J., Söderberg O., Paulus W., Ostman A. and Hasselblatt M. (2009). Platelet-derived growth factor receptor expression and activation in choroid plexus tumors. Am. J. Pathol. 175, 1631-1637.

Kubo T., Piperdi S., Rosenblum J., Antonescu C.R., Chen W., Kim H., Huvos A.G., Sowers R., Meyers P.A., Healey J.H. and Gorlick R. (2008). Platelet-derived growth factor receptor as a prognostic marker and a therapeutic target for imatinib mesylate therapy in osteosarcoma. Cancer 112, 2119-2129.

Lederle W., Linde N., Heusel J., Bzyl J., Woenne E.C., Zwick S., Skobe M., Fabian K., Fusenig N.E. and Mueller MM. (2010). Platelet-derived growth factor-B normalizes micromorphology and vessel function in vascular endothelial growth factor-A-induced squamous cell carcinomas. Am. J. Pathol. 176, 981-994.

Levine R.A.(2002). Overexpression of the sis oncogene in a canine osteosarcoma cell line. Vet. Pathol. 39,411-412 .

Liu Y.C., Chen S.C., Chang C., Leu C.M. and Hu C.P. (1996). Platelet-derived growth factor is an autocrine stimulator for the growth and survival of human esophageal carcinoma cell lines. Exp. Cell. Res. 228, 206-211.

Lokker N.A., Sullivan C.M., Hollenbach S.J., Israel M.A. and Giese N.A. (2002). Platelet-derived growth factor (PDGF) autocrine signaling regulates survival and mitogenic pathways in glioblastoma cells: evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. Cancer Res. 62, 3729-3735.

Lucas D.R. (2009). Angiosarcoma, radiation-associated angiosarcoma, and atypical vascular lesion. Arch. Pathol. Lab. Med. 133, 1804-1809.

Magnusson P.U., Looman C., Ahgren A., Wu Y., Claesson-Welsh L. and Heuchel R.L. (2007). Platelet-derived growth factor receptor-beta constitutive activity promotes angiogenesis *in vivo* and *in vitro*. Arterioscler. Thromb. Vasc. Biol. 27, 2142-2149.

Mark R.J., Poen J.C., Tran L.M., Fu Y.S. and Juillard G.F. (1996).

Angiosarcoma. A report of 67 patients and a review of the literature. Cancer 77, 2400-2406,

Matei D., Kelich S., Cao L., Menning N., Emerson R.E., Rao J., Jeng M.H. and Sledge G.W. (2007). PDGF BB induces VEGF secretion in ovarian cancer. Cancer Biol. Ther. 12, 1951-1959.

McCarthy C.J., O'Brien G.C., Cummins R.J., Kay E.W. and Broe P.J. (2010). GIST with a twist-up regulation of PDGF-B resulting in metachronous gastrointestinal stromal tumor and dermatofibrosarcoma protuberans. J. Gastrointest. Surg. 14, 398-403.

Mobini N. (2009). Cutaneous epithelioid angiosarcoma: a neoplasm with potential pitfalls in diagnosis. J. Cutan. Pathol. 36, 362-369.

Nakagawa M., Naruko T., Ikura Y., Komatsu R., Iwasa Y., Kitabayashi C., Inoue T., Itoh A., Yoshiyama M. and Ueda M. (2010). A decline in platelet activation and inflammatory cell infiltration is associated with the phenotypic redifferentiation of neointimal smooth muscle cells after bare-metal stent implantation in acute coronary syndrome. J. Atheroscler. Thromb. 17, 675-687.

Oksanen A. (1978). Haemangiosarcoma in dogs. J. Comp. Pathol. 88, 585-595.

Petti L., Nilson L.A. and DiMaio D. (1991).Activation of the platelet-derived growth factor receptor by the bovine papillomavirus E5 transforming protein. EMBO J. 10, 845-855.

Raica M. and Cimpean A.M. (2010). Platelet-derived growth factor (PDGF)/PDGF receptors (PDGFR) axis as target for antitumor and antiangiogenic therapy. Pharmaceuticals 3, 572-599.

Sabattini S. and Bettini G.J.(2009). Immunohistochemical analysis of heamangiosarcoma and heamangioma. J. Comp. Pathol. 140, 158-168.

Tamburini B.A., Trapp S., Phang T.L., Schappa J.T., Hunter L.E. and Modiano J.F. (2009). Gene expression profiles of sporadic canine hemangiosarcoma are uniquely associated with breed. PLos ONE 20, 5549.

Thamm D.H. (2007). Hemangiosarcoma. In: Small animal clinical oncology. 4th ed. Withrow S.J. and Vail D.M. (eds). Saunders. St. Louis, Missouri, USA. pp 785-795.

Tokuyama W., Mikami T., Masuzawa M. and Okayasu I. (2010). Autocrine and paracrine roles of VEGF/VEGFR-2 and VEGF-C/VEGFR-3 signaling in angiosarcoma of the scalp and face. Human Pathol. 41, 407-414.

Vassbotn F.S., Havnen O.K., Heldin C.H. and Holmsen H. (1994). Negative feedback regulation of human platelets via autocrine activation of the platelet-derived growth factor a-receptor. J. Biol. Chem. 269, 13874-13879.

Yonemaru K., Sakai H., Murakami M., Yanai T. and Masegi T. (2006). Expression of vascular endothelial growth factor, basic fibroblast growth factor, and their receptors (flt-1, flk-1, and flg-1) in canine vascular tumors. Vet. Pathol. 43, 971-980.

Zhang X., Rong T.H., Zhang Y., Long H., Fu J.H., Ling P., Zhang L.J., Yang M.T., Zeng C.G., Ma G.W., Su X.D., Li X.D., Wang J.Y., Wen Z.S. and Zhao J.M. (2006). Expression and significance of c-Kit and platelet-derived growth factor receptor-beta (PDGFR-beta) in esophageal carcinoma. Ai Zheng 25,92-95.

Accepted December 14, 2011