J. Comp. Path. 2014, Vol. 151, 322–328

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER
www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Platelet-derived Growth Factors and Receptors in Canine Lymphoma

A. Aricò*, E. Guadagnin*, S. Ferraresso*, M. E. Gelain*, S. Iussich†, B. C. Rütgen‡, S. Mazzariol*, L. Marconato§ and L. Aresu*

** Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16, 35020 Agripolis, Legnaro, PD, Italy, † Department of Veterinary Science, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco, TO, Italy, ‡ Clinical Pathology, Department of Pathobiology, University of Veterinary Medicine of Vienna, Veterinärplatz 1, 1210 Vienna, Austria and § Centro Oncologico Veterinario, via San Lorenzo 1-4, 40037 Sasso Marconi, BO, Italy*

Summary

Platelet-derived growth factors (PDGFs) belong to a family of polypeptide growth factors that signal through cell surface tyrosine kinase receptors to stimulate growth, proliferation and differentiation. Platelet-derived growth factor receptors (PDGFRs) are also considered important targets for specific kinase inhibitors in the treatment of several human tumours. The aim of this study was to investigate the role of PDGF-A, PDGF-B, PDGFR-α and PDGFR-β in canine lymphoma by determining gene and protein expression in lymph nodes of dogs with diffuse large B-cell lymphoma (DLBCL), peripheral T-cell lymphoma (PTCL), T-lymphoblastic lymphoma (T-LBL) and in healthy control dogs. One lymph node was also studied at the end of therapy in a subset of dogs in remission for DLBCL. In controls, PDGF-A, PDGFR-α and PDGFR-β mRNA levels were significantly higher than in DLBCLs, PTCLs and T-LBLs. However, PDGFR-α and PDGFR-β were minimally expressed by lymphocytes and plasma cells in normal lymph nodes as determined by immunohistochemistry, while neoplastic B and T cells showed the highest score ($P < 0.05$). This discordant result may be compatible with the constitutive expression of these molecules by endothelial cells and fibroblasts in normal lymph nodes, thereby influencing gene expression results. Furthermore, these cells were not included in the immunohistochemical analysis. Similarly, dogs with DLBCL that were in remission at the end of therapy showed significantly higher gene expression of PDGFs and receptors than at the time of diagnosis and with an opposite trend to the protein assay. PDGF-B protein and mRNA were overexpressed in PTCLs and T-LBLs when compared with DLBCLs and controls ($P < 0.05$). Additionally, there was a correlation between protein expression of PDGF-B and both PDGFRs in PTCLs and T-LBLs, suggesting an autocrine or paracrine loop in the aetiology of aggressive canine T-cell lymphomas. These data provide a rationale for the use of PDGFR antagonists in the therapy of aggressive T-cell lymphomas, but not in DLBCLs.

Keywords: angiogenic factors; canine lymphoma; platelet-derived growth factor; therapy

Introduction

Lymphoma, the most common haemopoietic tumour in dogs, includes numerous subtypes with varying morphology, pathophysiology and clinical features (Ponce *et al.*, 2010; Valli *et al.*, 2011; Aresu *et al.*, 2013). Dogs most commonly develop aggressive multicentric lymphoma, which shares some features with human non-Hodgkin lymphoma (hNHL) (Marconato *et al.*, 2013). Emerging data on the pro-angiogenic properties of lymphoma cells and the mechanisms of vascular assembly suggest that angiogenesis is highly relevant to the biology and therapy of hNHL (Moehler *et al.*, 2003; Ruan *et al.*, 2013). Several studies have demonstrated that cytokines involved in angiogenesis play an important role in the pathogenesis of hNHL, contributing to its progression in an autocrine and paracrine fashion (Pedersen *et al.*, 2005; Labidi *et al.*, 2010). In this context, platelet-derived growth factors (PDGFs)

Correspondence to: A. Aricò (e-mail: arianna.arico@unipd.it).

0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.07.001

are a pleotropic family of peptide growth factors that signal through cell surface tyrosine kinase receptors (TKRs) and stimulate various cellular functions including growth, proliferation and differentiation (Heldin and Westermark, 1999).

The PDGF pathway has been described in a wide range of human tumours, including T-cell lymphomas (Piccaluga *et al.*, 2005). The biological role of PDGF signalling can vary from autocrine stimulation of cancer cell growth to subtler paracrine interactions involving adjacent stroma and vasculature. Experimental studies have shown that the concomiant expression of PDGF ligands and receptors by the same neoplastic cells contributes to cancer progression by creating an autocrine loop (Heldin and Westermark, 1999). PDGFs may also modulate the expression of other angiogenic factors such as vascular endothelial growth factor (VEGF), whose receptors (Flt-1, KDR/Flk-1 and Flt-4) are also classified as TKRs (Halper, 2010). Recently, we suggested a potential role of VEGF in the pathogenesis of canine lymphoma, while the role of PDGF and its receptors is still unknown (Aresu *et al.*, 2012; Aricò *et al.*, 2013).

The aim of this study was to investigate the gene and protein expression of PDGF-A, PDGF-B, platelet-derived growth factor receptor (PDGFR)-α and PDGFR-β in canine B-cell and T-cell lymphomas. Two canine lymphoma cell lines were also tested to elucidate the possible involvement of PDGFs and PDGFRs without the confounding influence of the tumour microenvironment.

Materials and Methods

Case Selection and Tissue Sampling

Dogs were enrolled in the study after complete staging work-up, including surgical removal of an enlarged lymph node for histopathological and immunohistochemical diagnosis according to the guidelines of the World Health Organization (Valli *et al.*, 2011). Control lymph nodes were obtained from healthy adult dogs. For the purposes of the study, tumour samples were immediately divided into aliquots and stored for different analytical techniques. For histological examination and immunohistochemistry (IHC), tissues were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. For RNA isolation, aliquots of approximately 100 mg of neoplastic tissue were immersed in RNAlater® solution (Applied Biosystems, Foster City, California, USA) and stored at −20°C until used.

Owners of dogs with lymphoma were offered treatment of their animals with multidrug chemotherapy. For selected dogs, lymph node excision was also performed at the end of the chemotherapeutic protocol and/or at the time of relapse. The study was approved by the University Ethics Committee (protocol 20085MSFH2) and written consent was obtained from all owners.

Cell Lines

Canine cell lines used in this study as positive controls for B- and T-cell lymphomas included the B-cell lymphoma cell line CLBL-1 (Rütgen *et al.*, 2010) and the T-cell lymphoma cell line OSW (Kisseberth *et al.*, 2007). The cell culture conditions were described previously by Rütgen *et al.* (2010). For quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR), cells were harvested from the cell culture flask and washed twice in phosphate buffered saline without Mg^{2+} and Ca^{2+} (1× PBS) (PAA, Pasching, Austria). Aliquots of 5×10^6 cells were pelleted, supernatant was discarded and the pellets were frozen at −80°C. For immunocytochemical examination, the cells were harvested from cell culture, washed twice in PBS and aliquots of 5×10^5 cells were used for cytospin preparation.

Quantitative Reverse Transcriptase Polymerase Chain Reaction

Total RNA was isolated from tissue samples using TRIzol® (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. Total RNA concentration and quality were measured with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA). First-strand cDNA was synthesized from 300 ng of total RNA using Superscript II (Invitrogen) according to the manufacturer's protocol. The generated cDNA was used as the template for quantitative real-time RT-PCR (qRT-PCR) in a LightCycler 480 (Roche Diagnostics, Basel, Switzerland), using standard PCR conditions. The qRT-PCR reactions consisted of 5 μl of Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen), 0.3 μl of forward and reverse primers (10 μM) (the primer combinations and final concentrations were optimized during assay setup) and 2.5 μl of diluted (1 to 100) cDNA. The primers (Table 1) were designed using Primer Express 2.0 (Applied Biosystems, Carlsbad, California, USA). Calibration curves using a 7-fold serial dilution (1 in 2) of a cDNA pool revealed PCR efficiencies near 2.0 and error values <0.2. Canine transmembrane BAX inhibitor motif containing 4 (*CGI-119*) was chosen as reference gene for the absence of pathological state-dependent differences in mRNA expression (Aricò *et al.*, 2013). The ΔΔCt method (Livak and Schmittgen, 2001) was used for the relative