J. Comp. Path. 2016, Vol. ■, 1–9

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

www.elsevier.com/locate/jcpa

NEOPLASTIC DISEASE

Analysis of Obesity-Related Factors and their Association with Aromatase Expression in Canine Malignant Mammary Tumours

J.-I. Shin, H.-Y. Lim, H.-W. Kim, B.-J. Seung, J.-H. Ju and J.-H. Sur

Department of Veterinary Pathology, Small Animal Tumour Diagnostic Center, College of Veterinary Medicine, Konkuk University, 120 Neuengdong-ro, Gwangjin-gu, Seoul, Republic of Korea

Summary

This study was designed to investigate the role of obesity in canine malignant mammary tumours (CMMTs), by assessing aromatase expression and the regulatory roles of immune mediators such as cyclo-oxygenase-2 (COX2), prostaglandin E_2 (PGE_2), nuclear factor kappa beta (NF-κB), hypoxia inducible factor-1α (HIF-1α) and adipokines (i.e. leptin) in lean, optimal body weight, overweight and obese animals. Clinicopathological data, including the breed, body weight, body condition score and age and neutering status, were collected, together with histopathological characteristics (i.e. histological types, grading and lymphatic invasion). To determine the expression of each factor, immunohistochemistry was conducted with 60 samples of malignant CMMTs. CMMTs from overweight and obese animals had significantly elevated levels of PGE_2, and aromatase expression correlated significantly with PGE_2, NF-κB and leptin expression. However, no significant difference was observed in terms of histopathological characteristics. The results suggest that PGE_2, a known obesity-related immune mediator, could be upregulated in CMMTs from overweight and obese animals. In addition, PGE_2, NF-κB and leptin influenced the expression of aromatase, as observed in women.

Keywords: aromatase; canine mammary tumour; obesity; obesity-related factors

Introduction

The incidence of human obesity is increasing and it is estimated that globally one-third of human adults were overweight or obese in 2013 (Ng *et al.*, 2014). Epidemiological studies show that obesity is associated with cardiovascular, endocrine, metabolic and other chronic diseases (Vucenik and Stains, 2012). Additionally, obesity is related to several cancers, especially breast cancer in postmenopausal women in association with increased oestrogen levels (Endogenous Hormone and Breast Cancer Collaborative Group, 2003). Similarly, studies conducted in the USA and Australia reported the proportion of overweight and obese dogs was 34.1% (Lund *et al.*, 2006) and 41.1% (McGreevy *et al.*, 2005), respectively. In addition, the link between obesity and the incidence of canine mammary carcinoma has been reported, with some studies suggesting that obesity at an early age is associated with mammary tumours (Sonnenschein *et al.*, 1991), while others observed no association with obesity at the time of diagnosis with mammary tumours (Philibert *et al.*, 2003). Further studies are therefore required.

Obesity is often regarded as a low-grade, chronic inflammatory process, because adipocytes can release saturated fatty acids that mediate inflammatory signals (Milanski *et al.*, 2009) and an excess of adipose tissue could increase the levels of inflammatory mediators. Although the definitive role of obesity in tumourigenesis remains unclear, some studies suggest several mechanisms linking obesity and cancer,

Correspondence to: J.-H. Sur (e-mail: jsur@konkuk.ac.kr).

0021-9975/$ - see front matter

http://dx.doi.org/10.1016/j.jcpa.2016.05.005

Please cite this article in press as: Shin J-I, et al., Analysis of Obesity-Related Factors and their Association with Aromatase Expression in Canine Malignant Mammary Tumours, Journal of Comparative Pathology (2016), http://dx.doi.org/10.1016/j.jcpa.2016.05.005

including effects of adipokines (Lorincz and Sukumar, 2006) and inflammatory mediators (Simpson and Brown, 2013a), which dysregulate the tissue microenvironment.

Despite the decreased production of oestrogen in postmenopausal women, local oestrogen can be produced within adipose tissues and the tumour itself via activity of the enzyme aromatase (O'Neill *et al.*, 1988; Harada, 1997). Aromatase, which converts androgen to oestrogen by consecutive hydroxylation, is an enzyme that belongs to the cytochrome P450 family and is encoded by the *CYP19A1* gene (Bulun *et al.*, 2005). Recent studies suggested that aromatase expression can be regulated by various inflammatory mediators such as cyclo-oxygenase-2 (COX2), prostaglandin E_2 (PGE_2) and nuclear factor kappa beta (NF-κB), which act on tissue-specific promoters on the *CYP19A1* gene (Subbaramaiah *et al.*, 2012; Vucenik and Stains, 2012; Simpson and Brown, 2013b).

It has been reported that accumulation of adipocytes could result in a hypoxic microenvironment by impeding blood supply, thereby inducing hypoxia inducible factor-1α (HIF-1α) (Ye, 2009). In addition to accumulated adipose tissue evoking a hypoxic environment around tumours, it has been suggested that cancer cells might further express increased levels of HIF-1α to allow survival in challenging conditions (Khan *et al.*, 2013). HIF-1α can recruit macrophages, which produce PGE_2, interleukin (IL)-6 and tumour necrosis factor (TNF)-α in adipose tissues, thereby inducing aromatase expression (Cancello *et al.*, 2005; Irahara *et al.*, 2006) and impacting on the regulation of adipokines (Hosogai *et al.*, 2007).

A recent study revealed that HIF-1α and PGE_2 could collaboratively upregulate aromatase expression in breast adipose cells (Samarajeewa *et al.*, 2013), and PGE_2 can influence the stabilization and nuclear localization of HIF-1α in cancer cell lines (Liu *et al.*, 2002).

In the breast cancer cell line MCF-7, leptin has the ability to reinforce the expression of aromatase through the AP-1 motif, the binding site of AP-1 transcription factors that control various cellular pathways, on the promoter (Catalano *et al.*, 2003). Moreover, plasma leptin levels in postmenopausal patients with breast cancer correlate with total body aromatization, which represents the conversion of the androgen precursor into oestrogen in peripheral tissues, particularly adipose tissue, suggesting that leptin can affect aromatase activity *in vivo* (Geisler *et al.*, 2007).

In the veterinary research literature, some studies have examined the role of aromatase in mammary gland pathology (Subbaramaiah *et al.*, 2011; Lim *et al.*, 2015a), but the identification of the factors involved in the regulation of aromatase expression in canine malignant mammary tumours (CMMTs) has not been fully elucidated.

The aims of this study were (1) to analyze the expression of aromatase, inflammatory mediators (i.e. COX2, PGE_2, and NF-κB), HIF-1α and leptin in CMMTs in light of clinicopathological features and tumour histological characteristics, and (2) to determine the effects of these factors on aromatase expression.

Materials and Methods

Tissue Samples and Group Assignment

Formalin-fixed and paraffin wax-embedded tissue samples of CMMTs were selected from the archive of the Department of Veterinary Pathology, Konkuk University, Animal Teaching Hospital, Seoul, Korea. Samples had been submitted between 2012 and 2015. For each case selected, clinicopathological features had been recorded, including the breed, age, neuter status and body condition score (BCS) or body weight (BW). The specimens were randomly divided into two groups based on the body weight of the dog: lean or optimal body weight dogs (group 1; $n = 30$) and overweight or obese dogs (group 2; $n = 30$).

Group 1 dogs had a BCS of either 2 (below the minimal ideal BW of the breed) or 3 (in the ideal range of BW of the breed). Group 2 dogs had a BCS of 4 (over the maximal ideal BW of the breed) or 5 (obese or >15% of the maximal ideal BW of the breed) (Simpson *et al.*, 1993).

Histopathological Analysis

Haematoxylin and eosin (HE)-stained sections were examined to determine the histological type and grade of the CMMTs according to Goldschmidt *et al.* (2011). Lymphatic invasion was recorded when tumour cells were observed in lymphatic vessels in the tissue section. Histopathological analysis was conducted by two pathologists in a blinded fashion and then rechecked by the corresponding author.

Immunohistochemistry

To determine the expression of aromatase, PGE_2, COX2, NF-κB, HIF-1α and leptin, immunohistochemistry (IHC) was performed using primary antibodies specific for each factor. Sections (4 μm) were placed in a drying oven for 1 day and then dewaxed in xylene and rehydrated through a series of graded ethanols. Sections were treated with H_2O_2 3% in phosphate buffered saline (PBS; pH 7.4, 137 mM

Please cite this article in press as: Shin J-I, et al., Analysis of Obesity-Related Factors and their Association with Aromatase Expression in Canine Malignant Mammary Tumours, Journal of Comparative Pathology (2016), http://dx.doi.org/10.1016/j.jcpa.2016.05.005