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NEOPLASTIC DISEASE

Canine Splenic Haemangiosarcoma: Influence of Metastases, Chemotherapy and Growth Pattern on Post-splenectomy Survival and Expression of Angiogenic Factors

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Summary

Splenic haemangiosarcomas (HSAs) from 122 dogs were characterized and classified according to their patterns of growth, survival time post splenectomy, metastases and chemotherapy. The most common pattern of growth was a mixture of cavernous, capillary and solid tumour tissue. Survival time post splenectomy was independent of the growth pattern; however, it was influenced by chemotherapy and metastases. Immunohistochemical assessment of the expression of angiogenic factors (fetal liver kinase-1, angiopoietin-2, angiopoietin receptor-2 and vascular endothelial growth factor A) and conventional endothelial markers (CD31, factor VIII-related antigen) revealed variable expression, particularly in undifferentiated HSAs. Therefore, a combination of endothelial markers should be used to confirm the endothelial origin of splenic tumours.

Keywords: angiogenesis; dog; haemangiosarcoma; survival time

Introduction

Haemangiosarcomas (HSAs) are highly malignant tumours of endothelial origin. They may originate from any vascular site, but the most frequent locations in dogs are the spleen, the right atrium and the skin (Locke and Barber, 2006). The auricular appendage, liver, lungs and kidneys are further recognized primary sites (Brown *et al*., 1985; Pulley and Stannard, 1990; Goldschmidt and Hendrick, 2002). HSAs are common in dogs (Hosgood, 1987; Johnson *et al*., 1989) and are rarely reported in other species including cats, cattle, sheep and horses (Goldschmidt and Hendrick, 2002). An increased incidence of HSA in man has been shown with exposure to insecticides and irradiation (Paik and Komorowski, 1976; Restucci *et al*., 2004) as well as vinyl chlorides (Block, 1974). However, primary malignant vascular tumours are rare in people and the prevalence is much lower than in dogs (Oksanen, 1978). Benjamin *et al*. (1975) suggested that inhalation of radionuclides could trigger canine HSAs, but Oksanen (1978) found no such correlation between the development of these tumours and environmental factors.

Canine splenic HSAs occur mainly in older animals. A predisposition is described for dogs of the German shepherd (Oksanen, 1978; Brown *et al*., 1985; Johnson *et al*., 1989) and golden and Labrador retriever breeds (Spangler and Culbertson, 1992; Clifford *et al*., 2000; Schultheiss, 2004; Christensen *et al*., 2009). The boxer, Bernese mountain dog, German pointer and flat coated retriever are also breeds with a high relative risk (Moe *et al*., 2008).

Microscopically, HSAs consist of irregular vascular channels ('capillary' growth pattern) or expanded caverns ('cavernous' growth pattern), which are lined

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by variably differentiated neoplastic endothelial cells (Pulley and Stannard, 1990; Goldschmidt and Hendrick, 2002; Maxie and Robinson, 2007). So-called 'honeycomb' or 'spongiform' growth patterns can be found in human HSAs and appear to be equivalent to the cavernous pattern in animals, but with smaller spaces or clefts (Falk *et al*., 1993). Growth characterized by solid sheets ('solid' growth pattern) is seen rarely in animals. These solid HSAs represent a diagnostic challenge as they can mimic other sarcomas (Bettini *et al*., 2001; Yonemaru *et al*., 2006; Gamlem and Nordstoga, 2008).

The morphology of neoplastic endothelial cells in animal HSAs is generally heterogeneous. Spindle-shaped cells with round, oval or pleomorphic nuclei, prominent nucleoli and a low nuclear to cytoplasmic ratio, as well as moderate to abundant, basophilic and usually vacuolated cytoplasm can be detected (Pulley and Stannard, 1990; Goldschmidt and Hendrick, 2002; Bertazzolo *et al*., 2005). 'Epithelioid' HSAs with polygonal, histiocytic cells are described in dogs, horses and cows, arising particularly in the subcutis, skeletal muscles and lungs (Machida *et al*., 1998; Warren and Summers, 2007). Additionally, small monomorphic spindle-shaped cells with high nuclear to cytoplasmic ratios, round to oval nuclei, coarse chromatin and indistinct nuclei are also observed in canine HSAs (Bertazzolo *et al*., 2005).

In general, the prognosis for dogs with HSAs is poor. Those animals with tumours localized in the skin have longer median survival times (Ward *et al*., 1994). The reported median survival times for dogs with splenic HSA range from 19 days to 240 days (Johnson *et al*., 1989; Ogilvie *et al*., 1996; Spangler and Kass, 1997; Wood *et al*., 1998). Several factors have been proposed to be of prognostic value. Clinical staging appears not to be a significant indicator of survival time (Brown *et al*., 1985; Johnson *et al*., 1989; Wood *et al*., 1998), but administration of chemotherapy extends survival time relative to cases treated only by splenectomy (Ogilvie *et al*., 1996; Wood *et al*., 1998; Sorenmo *et al*., 2004). Naka *et al*. (1996) suggested that the survival time of people with splenic HSA depends on the mitotic rate of the neoplastic endothelial cells. There is controversy as to whether the differentiation of the tumour influences prognosis in dogs (Ogilvie *et al*., 1996; Dahl *et al*., 2008). However, to our knowledge, there are no studies that have investigated the correlation between the histological growth pattern of canine splenic HSAs and survival time after splenectomy.

Solid or poorly differentiated HSAs can mimic other sarcomas and so immunohistochemistry (IHC) using antibodies specific for the endothelial markers von Willebrand factor (vWF also called factor VIII-related antigen) and CD31 [also called platelet endothelial cell adhesion molecule (PECAM)] should be a useful diagnostic tool. However, both human and canine HSAs are often characterized by a minimal or absent expression of vWF (von Beust *et al*., 1988; Falk *et al*., 1993; Machida *et al*., 1998; Gamlem and Nordstoga, 2008). As tumour growth depends on angiogenesis (Folkman, 1971), angiogenic factors are also likely to be important in HSA (Plendl, 2000; Conway *et al*., 2001). Such factors include vascular endothelial growth factor A (VEGF A) and angiopoietin-2 (Ang-2) (Berse *et al*., 1992; Ferrara *et al*., 1992; Dvorak, 2000) and their receptors VEGF-R2 (the receptor for VEGF A; also called fetal liver kinase-1; Gale and Yancopoulos, 1993) and Tie-2 (the receptor for Ang-2), which are located on endothelial cells (Hanahan, 1997).

The aims of the present study were (1) to determine whether the survival times of dogs with splenic HSA is influenced by the histological growth pattern or by the expression of angiogenic factors and their receptors within the tumours, and (2) to determine whether expression of angiogenic factors and their receptors is helpful in the diagnosis of solid or poorly differentiated HSAs.

Materials and Methods

Animals

Over an 8-year time span (2001–2009), 122 cases of splenic HSA were examined. The dogs ranged in age from 2 years to 14 years and were of different breeds (Table 1). Clinical outcome, date of splenectomy and date of death as well as chemotherapy and existing metastases were recorded. Six normal canine spleens from routine necropsy examinations served as controls for IHC.

Pathology

Directly after removal spleens were fixed in 4% neutral buffered formalin for 24–48 h. Thereafter, spleens were measured, assessed for colour and texture and serially sectioned (0.5 cm slices). Representative samples ($n = 1$–15) of each spleen were embedded in paraplast, sectioned (3–4 μm) and stained with haematoxylin and eosin (HE).

The tumours were classified according to the criteria of the World Health Organization (Hendrick *et al*., 1998). Each tumour was assigned to a specific histological growth pattern (Table 2). Neoplastic endothelial cells were characterized according to cellular morphology, nuclear to cytoplasmic ratio, number of mitoses per high-power field (×40 objective), nuclear content of chromatin and the number