J. Vet. Sci. (2004), **5**(2), 173–180

JOURNAL OF
Veterinary
Science

Canine biphasic synovial sarcoma: case report and immunohistochemical characterization

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The clinical, radiological and pathologic features of a biphasic synovial sarcoma in the left elbow joint of a two-year-old male Rottweiler are presented. The tumor showed positive immunoreactivity for vimentin, Epithelial Membrane Antigen (EMA), p53 and PCNA, while it was negative for the cytokeratin used, S-100, Rb and p21. Immunohistochemistry for EMA allowed the identification of epithelioid components of synovial sarcoma, and may, therefore, contribute in establishing a diagnosis of biphasic synovial sarcoma. Intratumoral variation in PCNA immunoreactivity was minimal, indicating that the various tumor components proliferate at more or less similar rates. Overall, the characterized immunohistochemical profile for canine synovial sarcoma, not defined previously, may provide clues to the histogenesis of the phenotypically mesenchymal and epithelial elements of the tumor, and may be of value in the differential diagnosis of challenging cases, decreasing the risk of under- and mis-diagnosis. Although more cases need to be studied to determine whether there is a consistent pattern of immunostaining in canine synovial sarcoma, its potential significance is discussed in relation to the histogenesis, molecular pathology and differential diagnosis of canine synovial sarcoma.

Keywords: Epithelial Membrane Antigen, dogs, immunohistochemistry, neoplasms, p53, radiology, pathology, biphasic synovial sarcoma

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Introduction

Tumors affecting joints are rare, and most are malignant rather than benign. Those reported in the veterinary literature are almost exclusively synovial sarcomas affecting dogs [2,37]. Synovial sarcoma has been reported infrequently in cats, cattle, horses and other species [33], although it accounts for about 8% of all soft-tissue sarcomas in humans [33]. In dogs, it most frequently involves the stifle and elbow, although other sites, including, for example, a rare case with bilateral hip joint involvement, have been reported [17].

Despite the assumed mesenchymal origin of the tumor and a morphological similarity with normal synovial tissue lining joints and tendon sheaths, the histogenesis of synovial sarcoma is not clearly defined [10,18,23]. Microscopically, it may be characterized by a monophasic or biphasic cellular pattern; the biphasic pattern is diagnostically more distinct and comprises of a sarcomatous component and an epithelioid component, which may form clefts and pseudoacini [25]. Synovial sarcoma may therefore resemble malignant fibrous histiocytoma, fibrosarcoma, giant-cell tumor of soft tissue or other tumors [5,31]. Consequently, it may present a diagnostic challenge for some pathologists and may be underdiagnosed, particularly atypical or monophasic variants, without the classic biphasic features, or cases with metaplastic bone formation or calcification zones. It is particularly important to differentiate synovial sarcomas from osteosarcomas, a defining feature of which is osteoid production by the malignant cells, because the latter tend to metastasize to the lungs and lymph nodes later in the clinical course of the disease [25,32].

Similarly, while the possible role of certain oncogenes and tumor suppressor genes [5], such as β-catenin [14] and p53 [28], in the pathogenesis of human synovial sarcoma has been reported, the molecular pathology of canine synovial sarcoma has not studied extensively or understood.

A specific immunohistochemical profile for canine synovial sarcoma has not been clearly defined previously, and reports on various epitopes are sparse [1]. The

development of such a profile may provide clues to the histogenesis of the phenotypically mesenchymal and epithelial elements of the tumor, while, at the same time, it may be of value in the differential diagnosis of challenging cases, decreasing the risk of under- and mis-diagnosis. Immunohistochemical detection of Epithelial Membrane Antigen (EMA), for example, may allow the identification of epithelioid components of human synovial sarcoma [27], although immunoreactivity to EMA has not been reported in canine tissues.

In the case presented here, the clinical, radiological and pathologic features of a biphasic synovial sarcoma in a young dog are described, the tumor is characterized using immunohistochemistry and histochemistry, and the immunohistochemical profile of the tumor is discussed in relation to the histogenesis, pathogenesis and differential diagnosis of canine synovial sarcoma.

Materials and Methods

A two-year-old male Rottweiler was referred to the University Veterinary Hospital, Universiti Putra Malaysia for evaluation of progressive lameness of the left forelimb of 4 months duration. Clinical, cytological and radiological examinations, and following the animal's euthanasia, the post mortem and histopathological examination were performed in a routine fashion.

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded, silane-coated slides employing a streptavidin-biotin-peroxidase protocol as described previously [15,21], counterstained with haematoxylin. The antibodies used were: vimentin (V9 antibody, 1 : 400 dilution), cytokeratin (MNF116, 1 : 800), S-100 (1 : 400), Proliferating Cell Nuclear Antigen (PCNA, PC-10, 1 : 800), Epithelial Membrane Antigen (EMA, E29) (all DAKO, Carpinteria, USA), p21 protein (NCL-WAF1, 1 : 30), Retinoblastoma susceptibility gene protein (NCL-Rb, 1 : 50, both Novocastra), and p53 protein (CM-1 antibody, 1 : 75, Signet Laboratories, USA). The slides were subjected to ten minutes of microwave heating (low setting) in a citrate buffer, pH 6. Positive controls were neoplastic or normal tissues known to contain the relevant epitope [7,36]. The primary antibody was substituted with non-immune sera or Tris buffer in the negative controls.

Results

History and clinical examination

A two-year-old male Rottweiler was referred to the University Veterinary Hospital, Universiti Putra Malaysia for evaluation of progressive lameness of the left forelimb of 4 months duration. It had no history of trauma and many veterinarians had treated it with different types of non-steroidal anti-inflammatory drugs, without improvement of the lameness. Upon physical examination, the dog was depressed, and having non-weight bearing lameness of the left fore limb. Severe muscle atrophy of the limb was observed. Pain was evident upon palpation and manipulation of the elbow joint. There was also evidence of soft tissue swelling around the joint. Neurological examination revealed no abnormalities.

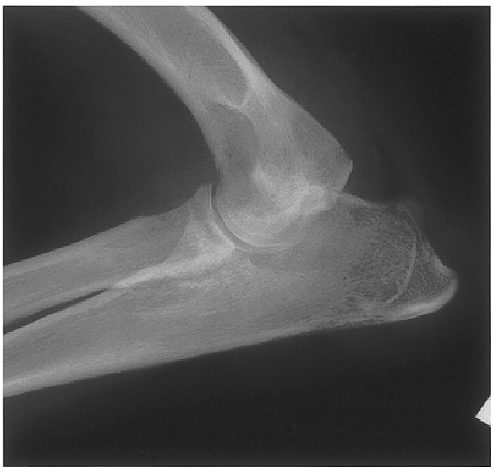

Fig. 1. Mediolateral radiographic view of the left elbow. Generalized reduced density of the distal humerus and also the proximal radius and ulna. The trabecular pattern of the olecranon is ill defined. Note the cortical destruction of the cranial border of medial epicondyle, the cranial border of lateral epicondyle, and the cortical destruction of the proximal cranial border of the radius.

Initial radiological examination

The mediolateral radiograph of the left elbow revealed generalized reduced density of the distal humerus and also the proximal radius and ulna. The trabecular pattern of the olecranon was ill defined. Cortical destruction of the cranial border of the medial epicondyle as well as the cranial border of the lateral epicondyle was observed. Cortical destruction of the proximal cranial border of the radius was also present (Fig. 1). The cranio-caudal view of the elbow showed that there was a focal rounded lytic area, approximately 2 cm in diameter, at the supratrochlear foramen of the humerus. Two foci of cortical destruction, of the lateral and medial epicondyles of the humerus respectively, were also observed (Fig. 2). There was evidence of soft tissue swelling in both radiographs. Thoracic radiography was unremarkable.

Management

Following this, fine needle aspiration was carried out, but, unfortunately, only numerous erythrocytes and few leucocytes were observed on cytological examination. No conclusive diagnosis was made. The tentative diagnoses at this point included synovial sarcoma, deep fungal infection and metastatic neoplasia. Recommendations to the owner included core biopsy of the lesion, amputation of the limb or

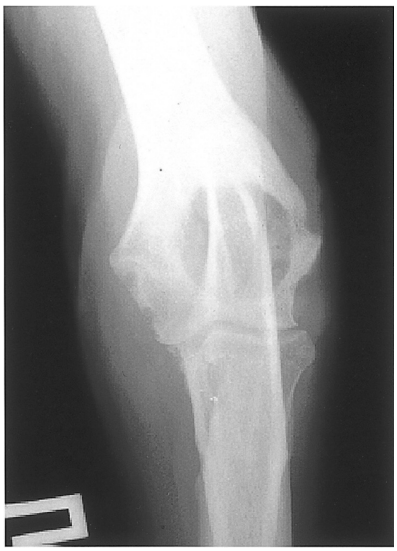

Fig. 2. Craniocaudal radiographic view of the left elbow. A focal rounded lytic area at the supratrochlear foramen of the humerus, and two foci of cortical destruction of both the medial and lateral epicondyles are shown.

euthanasia. In view of the guarded prognosis, the owner decided to bring the dog back home for a few days before euthanasia. Enrofloxacin (Baytril, Bayer, Germany) 5 mg/kg once a day and ketoprofen (Ketofen, Merial, France) 1 mg/kg once a day for 5 days were dispensed, to prevent secondary bacterial infection following the fine needle aspiration and to alleviate pain.

Clinical and radiological re-evaluation

After two weeks, the dog was presented again. The owner reported that there was no improvement of the dog's condition. Left elbow radiography was carried out again to determine the progression of the disease. The mediolateral view of the left elbow revealed that there was further cortical destruction of the cranial border of the medial and lateral epicondyle and the proximal cranial border of the radius (Fig. 3). The craniocaudal view radiograph showed that the focal lytic area had expended and that it had a diameter of about 2.5 cm (Fig. 4). The lateral epicondyle was nearly destroyed. Euthanasia was performed on the request of the owner. The reason given was that the owner could not bear having a three-legged dog and the agony over the possibility of the development of tumor metastases.

Gross pathology

The post-mortem examination was carried out immediately after euthanasia. Grossly the lesion extended from the distal humerus to the proximal radius and ulna. An irregular lobulated white mass, of approximately 2.5 cm in diameter, was found at the medial condyle of the humerus (Fig. 5). Another mass, hemorrhagic in appearance, was found in the elbow joint (Fig. 6). The cancellous bones of

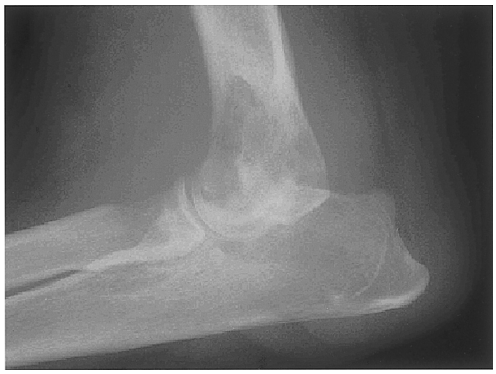

Fig. 3. Mediolateral radiographic view of the left elbow. Day 14: further cortical destruction of the cranial border of the medial and lateral epicondyle and the proximal cranial border of the radius.

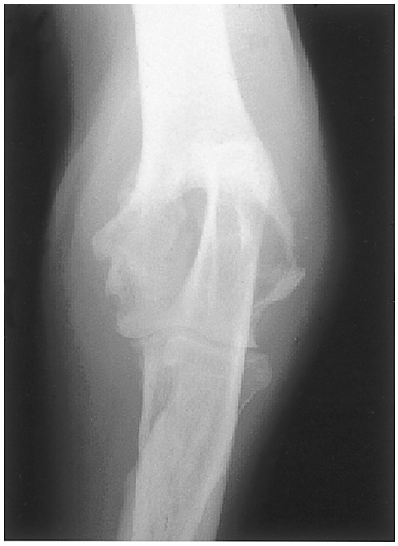

Fig. 4. Craniocaudal radiographic view of the left elbow. Day 14: The focal lytic area has expended; the lateral epicondyle is nearly destructed.

the distal humerus and olecranon were destroyed. The lungs were normal upon examination. There were no significant findings in other organ systems. Multiple tissue samples from different parts of the lesions and various organs were taken for histopathological examination.

Histopathology

Histopathologic examination was based on multiple tissue sections covering different parts of the tumor. The tumor appeared well circumscribed, unencapsulated and in intimate relationship to synovial structures. The mass was composed of highly pleomorphic cells, arranged in various morphological patterns. These mainly included cellular areas consisting of sheets of roundish to polygonal cells and areas consisting mostly of spindle cells arranged in a storiform pattern (Figs. 7 and 8).

In the former area, the dense stroma of cells did not

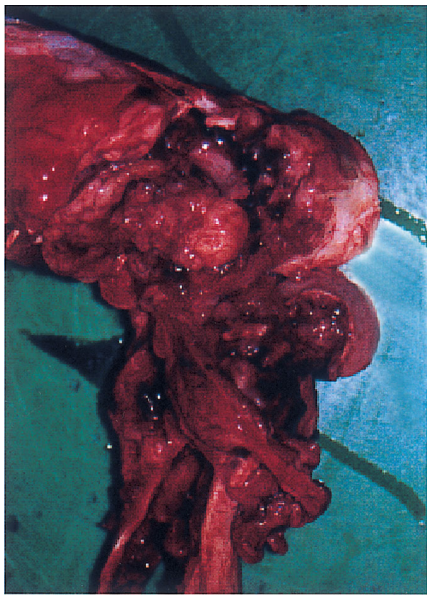

Fig. 5. Gross pathology: a lobulated mass at the medial condyle of the humerus.

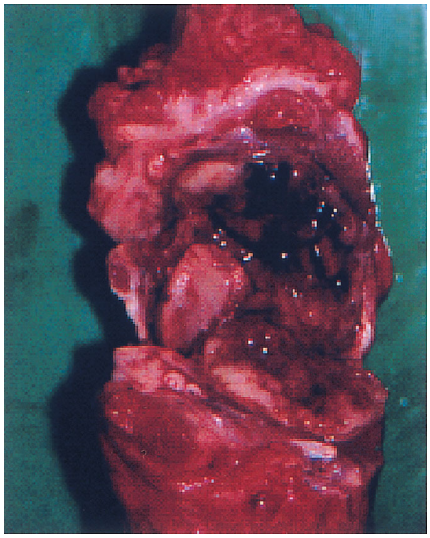

Fig. 6. Gross pathology: hemorrhagic mass found in the elbow joint.

produce any considerable amount of extracellular matrix, apart from small quantities of collagen. Small to medium-sized scattered necrotic foci covered approximately 30% of the area and were associated with hemorrhage. Cells were large, round, polygonal or, less frequently, spindle-shaped with distinct mauve cytoplasm and eccentric nuclei. The nuclear:cytoplasmic ratio was moderate. Cellular features included karyomegaly, anisokaryosis and bizarre mitoses; nuclei were round to oval in most cells or elongated in the spindle cells. Epithelioid cells with vesicular nuclei were occasionally encountered. A large proportion of cells had one or two prominent nucleoli and stippled chromatin

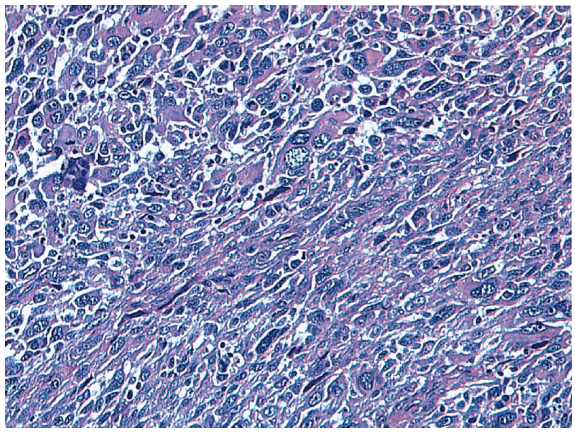

Fig. 7. Synovial sarcoma. Mixed tumor cell population, including spindle, epithelioid and multinucleated cells (H & E ×200).

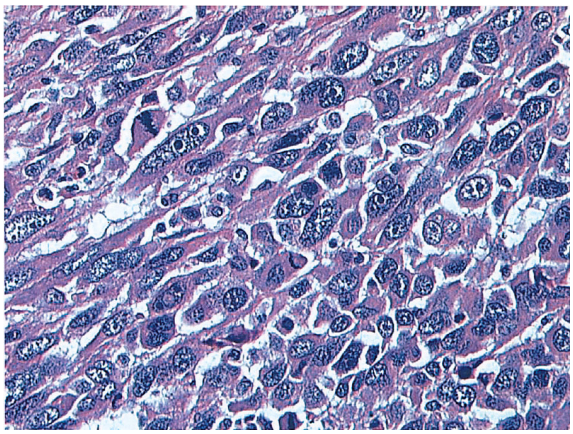

Fig. 8. Synovial sarcoma. Pleomorphic tumor cell population; cells with one or two prominent nucleoli, abnormal mitotic figures and apoptotic bodies are shown (H & E ×400).

pattern. There was a considerable number of bi- and tri-nucleated cells and giant mononucleated cells; the nuclei of the multi- and mono-nucleated cells were similar. Apoptosis was moderate while the mitotic index varied from 2 to 7 per high power field (average 3.3).

The latter areas of the tumor were reminiscent of malignant fibrous histiocytoma, characterized by whorling and streaming patterns of indistinctly bordered elongated cells and rather dense collagen fibers. Anisokaryosis was marked, mitotic index was lower in this area (2.5/high power field), while there were no necrotic foci.

In other areas, a mixed population of round or small fibroblast-like cells formed streams or packets of cells in a loose mucin-like matrix or collagen. A small number of slits and clefts lined by malignant cells were noticed. Mitoses, multinucleated and giant cells were few.

No osteoid or chondroid production were observed. There was evidence of osseous invasion by the tumor cells, including the medullary cavity. The lymph nodes examined

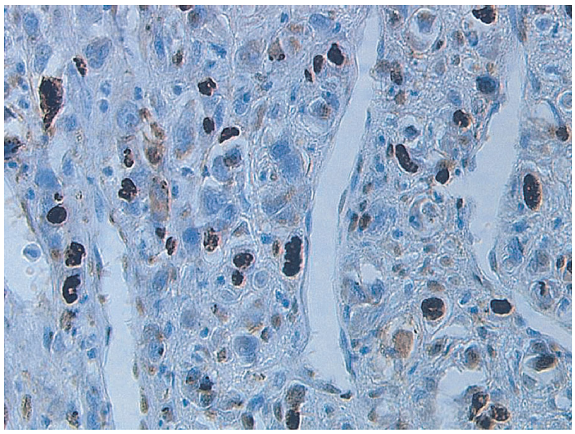

Fig. 9. Immunohistochemical localization of PCNA: intense nuclear staining of the majority of tumor cells. Streptavidin-biotin-peroxidase, Mayer's hematoxylin counterstain. ×400.

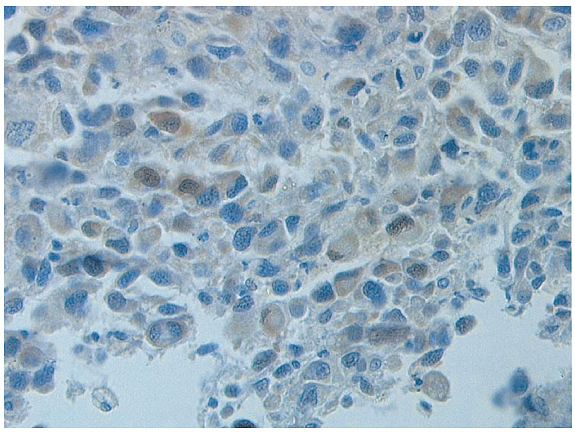

Fig. 10. Immunohistochemical localization of p53 protein: moderately intense nuclear staining of tumor cells. Streptavidin-biotin-peroxidase, Mayer's hematoxylin counterstain. ×200.

did not appear to be affected. The above elements allowed the diagnosis of biphasic synovial sarcoma to be established. Overall, based on the degree of nuclear pleomorphism (marked), the number of mitoses per 10 high power (400x) fields [21] and the proportion of tumor necrosis (<10% overall), the tumor was classified as Grade II[35].

Histochemistry and immunohistochemistry

Tumor sections stained with Alcian Blue allowed the detection of mucin in the interstitial matrix, while staining following a PAS reaction was unremarkable.

More than 80% of the tumor cells showed intense cytoplasmic staining for vimentin, while the majority of them also showed strong nuclear staining for PCNA (Fig. 9). p53 was detected immunohistochemically in 10-20% of tumor cells that showed moderate to intense nuclear staining (Fig. 9). Foci of cells positive to EMA were observed (Fig. 10). Tumor cells were uniformly negative to the cytokeratin

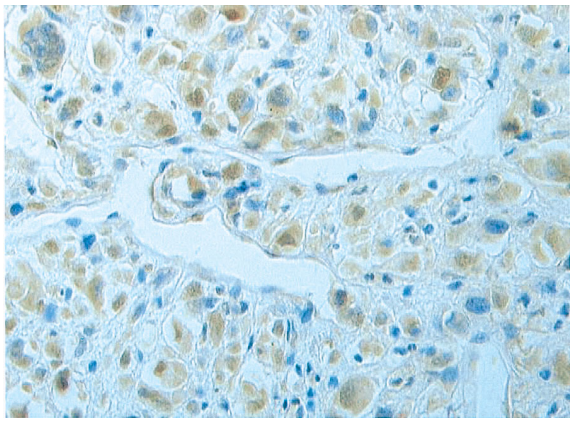

Fig. 11. Immunohistochemical localization of Epithelial Membrane Antigen: diffuse, moderately intense cytoplasmic staining of tumor cells. Streptavidin-biotin-peroxidase, Mayer's hematoxylin counterstain. ×400.

used, as well as to the Rb, p21 and S-100 antibodies (Fig. 11).

Discussion

Synovial sarcoma is a rare tumor in dogs [37], that may present histologically as a monophasic (mesenchymal) or a biphasic (mesenchymal and epithelial) variant. The histogenesis, pathogenesis and immunohistochemical profile of canine synovial sarcoma have not been clearly defined [10,18,23], and the veterinary literature on the subject is sparse [1]. In the case presented here, the clinical, radiological and pathological features of a biphasic synovial sarcoma in a young dog are described. The tumor is characterized using immunohistochemistry and histochemistry, and the derived immunohistochemical profile is discussed in relation to the histogenesis and differential diagnosis of canine synovial sarcoma.

Synovial sarcoma occurs mainly in male, middle-aged large breed dogs [22]; our patient, however, was only two years old. Progressive lameness is a clinical sign common to all cases. The duration of the lameness generally ranges from several weeks to months, although longer periods have been recorded [22]. In this case, the dog was progressively lame for 4 months and, upon presentation, it was not weight bearing, due to pain in the elbow joint.

Presence of a periarticular soft tissue mass may be the only radiographic finding and an ill-defined periosteal reaction with some cortical thinning may be the earliest detectable skeletal change [20]. This progresses to more obvious areas of trabecular and cortical lysis with poorly defined margins [11]. The tumor commonly crosses the joint, resulting in involvement of adjacent bones [22]. Beside the presence of periarticular soft tissue mass, the interesting radiographic features of this case include the

aggressiveness of the osteolytic lesion of the humerus and the cortical thinning of the epicondyles. The periosteal reaction was minimal.

Most patients with synovial sarcomas develop metastatic tumors [35]. Amputation, localized resection of the tumor and chemotherapy are the options of treatment for synovial sarcoma [35]. Amputation of the involved limb yields a better prognosis as most dogs have disease-free interval and survival time of more than 36 months. Local resection is invariably followed by recurrence at the site and then amputation is the appropriate recourse [35]. The outcome of chemotherapy in the treatment of synovial sarcoma in dogs is not good; although various chemotherapeutics have been reported to have efficacy as adjuvants in treating synovial sarcoma in human beings, there is only one report of a positive response to treatment in the dog, with a combination of cyclophosphamide and doxorubicin [34]. In the case presented here, however, the owner opted for euthanasia, despite the good prognosis for amputation, and the absence of radiographic evidence of lung metastasis of the tumor upon presentation.

On histopathological examination, osteoid production by the malignant cells, a defining feature of osteosarcoma, a tumor entity encountered much more frequently in practice, was not observed in our case. On examination of one tumor area alone, however, the dominant presence of a storiform pattern or multinucleated cells meant that the diagnosis of malignant fibrous histocytoma or giant cell tumor of bone could be entertained. When multiple sections were examined, however, the typical biphasic pattern of the tumor, combined with the clinical, radiological, and immunohistochemical findings, rendered the diagnosis of synovial sarcoma. The histochemical stain for Alcian Blue helped confirm the diagnosis.

The immunohistochemical profile of the tumor was determined, and was compatible with and suggestive of a diagnosis of synovial sarcoma. The positivity of the tumor cells for vimentin was indicative of their mesenchymal derivation [29]. Tumor cells were uniformly negative to the cytokeratin used. The above are in agreement with a recent study, in which all synovial sarcomas stained positive for vimentin and negative for cytokeratins [8]. Despite being negative to cytokeratin, foci of cells positive to Epithelial Membrane Antigen (EMA) were observed. EMA immunohistochemistry may allow the identification of epithelioid components of synovial sarcoma [27], and in our case, tends to confirm the biphasic nature of the tumor, although a diagnosis of osteosarcoma should not be ruled out on the basis of EMA immunoreactivity alone [16]. To our knowledge, this is the first time that positive immunoreactivity to EMA is reported in canine tissues.

S-100 is a protein that serves as a marker for bone tumors originating in the cartilage, the notochord and T-zone histiocytes and is also involved in the calcification of normal and neoplastic cartilage [4,24]. The fact that the present case was S-100 negative further supports our previous observations concerning the histogenesis of the tumor.

Proliferating Cell Nuclear Antigen (PCNA) acts as an auxiliary protein for DNA-polymerase delta and is increased in proliferating cells as opposed to mitotically quiescent cells [38]. It serves as a proliferation marker [6] and has been shown to be of prognostic value for a number of tumor types [26]. The majority of tumor cells in our case showed strong nuclear staining for PCNA. Interestingly, intratumoral variation in PCNA immunoreactivity was minimal, indicating that the various tumor components proliferate at more or less similar rates.

p53 was detected immmunohistochemically in 10-20% of tumor cells that showed moderate to intense nuclear staining. The p53 gene normally acts as a tumor suppressor gene [9]. p53 guards cells against replication when their genome is abnormal, by arresting the cell cycle and by either activating and regulating DNA repair, or by inducing programmed cell death (apoptosis) if genomic damage is excessive [9]. Because of its central role in the cell cycle and in carcinogenesis [9], p53 is the most frequently altered gene in human tumors [13]. Immmunohistochemical detection of p53 protein, as in the present case, demonstrates alterations in the p53 gene or product [19] which, therefore, appears to be involved in the pathogenesis of canine synovial sarcoma. However, the exact mechanisms of p53 involvement and progression toward a tumor-associated phenotype in synovial sarcoma would require further molecular studies in order to be elucidated.

Mutational inactivation of the retinoblastoma susceptibility gene (Rb) has been proposed as a crucial step in the formation of retinoblastoma and other tumors, including human synovial sarcoma and osteosarcoma [3,30]. p21 protein, the product of WAF1/CIP1 gene, is an inhibitor of cyclin-dependent kinases and a critical downstream effector in the p53 pathway. The expression of p21 in human neoplasms may be related to p53 functional status [12]. In our case, neither Rb nor p21 were expressed immunohistochemically, however, a role for them in the pathogenesis of synovial sarcoma cannot be ruled out.

The immunohistochemical profile of a case of canine synovial sarcoma was defined and its potential significance in relation to the molecular pathology and histogenesis of canine synovial sarcoma discussed. Overall, the case presented here was positive for vimentin, EMA, p53 and PCNA, while it was negative for the cytokeratin used, S-100, Rb and p21. Immunohistochemistry for Epithelial Membrane Antigen, in particular, may confirm the biphasic nature of the tumor, by allowing the identification of epithelioid components of synovial sarcoma, and may, therefore, contribute in establishing a diagnosis of biphasic synovial sarcoma. More cases will need to be studied by this technique, however, in order to determine whether there is a

consistent pattern of staining in synovial sarcomas of the dog and whether this pattern will be adequately specific to be of value in the differential diagnosis of synovial sarcoma.

Acknowledgments

We are grateful to Dr.S.Jasni, Faculty of Veterinary Medicine, Universiti Putra Malaysia; Professor W.F.Robinson and Dr.S.Yeomans, School of Veterinary Science, University of Queensland for constructive comments on histopathology, and Mr.M.Walsh, Department of Pathology, School of Medicine, University of Queensland for technical advice on EMA immunohistochemistry.

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