Abstract

Osteosarcoma is the most common bone tumour and one of the most malignant canine tumours. It bears a striking resemblance to human osteosarcoma, both clinically and histopathologically, and has served as an excellent model for its study.

The clinicopathological features of 172 canine osteosarcoma cases that occured in Australia were analysed and examined in relation to each other. Osteosarcomas were classified into histopathological subtypes according to the quality and quantity of the extracellular matrix produced, and into histopathological grades based on their mitotic index, degree of necrosis and nuclear pleomorphism. The clinicopathological relevance of tumour grading and the classification into subtypes were evaluated and established in osteosarcoma. The histopathological grade of the tumour was associated with the age of the animal, tumour location and subtype; metastatic tumours had significantly higher grade compared with non-metastatic osteosarcomas.

The initial diagnosis of osteosarcoma was revised in 20% of cases, which were reclassified into 15 other tumour entities, the most prominent being the multilobular tumour of bone and chondrosarcoma. The presence of non-neoplastic bone in the tumour, the rarity of specific pathological entities and/or their manifestation in unusual locations were the main reasons for misdiagnosis. Recognition of the varied appearance of osteosarcoma, of multilobular tumour of bone as a separate entity and, the differentiation of neoplastic from non-neoplastic bone in tumours may prevent confusion with other pathologic entities.

The clinicopathological value of the immunohistochemical expression of p53 tumour supressor gene protein was evaluated in 167 osseous tumours. p53 staining frequency and intensity in tumour cells was expressed as a p53 index. p53 index was significantly higher in osteosarcomas compared with other sarcomas, chondrosarcoma, multilobular tumour of bone and tumours misdiagnosed as osteosarcomas; in appendicular versus axial and in distal versus proximal osteosarcomas. A strong correlation was demonstrated between the p53 index and a range of clinicopathological parameters in osteosarcoma, including the tumour site,

histological grade and score, mitotic index, degree of tumour necrosis and pleomorphism. Chondroblastic osteosarcomas had significantly higher and telangiectatic osteosarcomas lower p53 index than osteosarcomas belonging to other histopathological subtypes, a fact that tends to reinforce the perception of them being distinct clinicopathological entities. Entire males had higher p53 index than neutered males. p53 index was higher in Rottweilers than Great Danes and Terriers, confirming breed susceptibilities to osteosarcoma. p53 index showed no association with age, primary or secondary site status, or the presence of metastases or other tumour types. The immunohistochemical examination for p53 may be used as an additional diagnostic tool and prognostic indicator for osseous tumours.

Matrix metalloproteinases (MMPs) are a family of enzymes implicated in the remodelling of extracellular matrix and in pathologic processes such as tumour invasion and metastasis in experimental cancer models and in human malignancies. Gelatin zymography and immunohistochemistry were employed to determine whether MMP-2 and MMP-9 are present in canine tumours and normal tissues, and whether MMP production correlates with clinicopathological parameters of prognostic importance. High levels of pro-MMP-9, pro-MMP-2 and active MMP-2 were detected in most tumours. Significantly higher MMP levels were measured in tumours than non-tumours and malignancies than benign tumours. Cartilaginous tumours produced higher MMP levels than non-sarcomatous malignancies, benign tumours and normal tissues, and significantly greater MMP-2 than osteosarcomas. Pro-MMP-9 production correlated with the histological grade of osteosarcomas. The 62kDa active MMP-2 was only detected in high-grade, p53 positive, metastatic malignancies. Zymography proved to be a sensitive and quantitative technique for the assessment of MMP presence, but requires fresh tissue; immunohistochemistry is qualitative and comparatively insensitive but could be of value in archival studies. MMP presence was shown in a range of canine tumours and their link to tumour type and grade was demonstrated for the first time. This study will provide baseline information necessary for the design of clinical trials targeting MMPs.

The demonstration of MMP involvement in canine neoplasms in our study made MMPs a promising therapeutic target. Canine osteosarcoma cell lines were produced and characterised based on their biochemical, histomorphological and ultrastructural

features, proliferation rate, MMP and immunocytochemical profile. The new cell lines maintained the characteristics of osteosarcoma and provide an excellent *in vitro* model that will allow the direct effects of novel therapeutic agents, such as matrix metalloproteinase inhibitors, on tumour growth to be quickly assessed.

Material from primary and cultured osteosarcoma was transplanted subcutaneously and intravenously into nude mice, to create a serial subcutaneous and lung xenotransplantation model of both the primary and secondary tumour. The tumours retained characteristics of the donor tumours, but were self-limiting and the overall take-up rate was low. Some evidence of retroviral involvement was found in the primary, cultured and xenotransplanted osteosarcomas investigated with electron microscopy, but not with RT-PCR.