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Characterizing Microscopical Invasion Patterns in Canine Mast Cell Tumours and Soft Tissue Sarcomas

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Summary

Stromal invasion is identified commonly in cutaneous malignancies; however, invasive patterns are defined inconsistently and their clinical relevance is uncertain. This study aimed to define objective, quantifiable histomorphological invasive patterns in low-grade canine mast cell tumours (MCTs) and grade I/II soft tissue sarcomas (STSs), and correlate invasive patterns with overall excisional status. Haematoxylin and eosin-stained glass slides prepared for routine histopathology of surgically-excised tumours from client-owned dogs were evaluated for invasion beyond their subgross edge, asymmetrical invasion, satellite lesions, lymphovascular invasion, perineurovascular growth, growth along fascial planes, intramuscular invasion and multicompartmental involvement. Digital histological tumour-free margins <1 mm in any direction were considered to represent an incomplete excision. Fifty-one dogs with 69 tumours (50 MCTs and 19 STSs) were included in the study. Invasion in both circumferential and deep directions was significantly greater in MCTs compared with STSs (exact 2-tailed $P < 0.0001$ circumferential; $P = 0.0095$ deep). Within the MCT group, circumferential invasion was greater than deep invasion ($P = 0.0076$). Multivariate logistic regression analysis found two variables that were significantly associated with incomplete MCT excision: intraoperative grossly normal circumferential surgical margin size (odds ratio of 0.776, 95% confidence interval: 0.651–0.925) and asymmetry invasion index (odds ratio of 1.318, 95% confidence interval: 1.039–1.671). These data may help create evidence-based strategies for planning surgical resections of cutaneous malignancies. Presence of asymmetrical microscopical invasion might prompt pathologists to perform more comprehensive surgical margin evaluation.

Keywords: dog; invasion; mast cell tumour; soft tissue sarcoma

Introduction

The two most common cutaneous and subcutaneous malignancies affecting dogs are grade II or 'low-grade' mast cell tumours (MCTs) and grades I and II soft tissue sarcomas (STSs) (Kuntz *et al.*, 1997; Dobson *et al.*, 2002; Dennis *et al.*, 2011; Sledge *et al.*, 2016; Terry *et al.*, 2017). These tumours are typically treated by surgical excision with similar grossly normal surgical margin resection recommendations; histologically-confirmed complete excision is associated with improved patient outcomes (Kuntz *et al.*, 1997; Murphy *et al.*, 2004; Simpson *et al.*, 2004; Stefanello *et al.*, 2008; McSporran, 2009; Scarpa *et al.*, 2012). While some degree of stromal invasion is common in both tumour types, contemporary grading schemes for MCT and STS growth patterns do not take into account microscopical growth

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patterns, despite heterogeneity within and between tumour grades (Patnaik *et al.*, 1984; Kuntz *et al.*, 1997; Kiupel *et al.*, 2011). Invasive growth patterns have prognostic significance in a variety of human neoplasms, including squamous cell carcinoma, urothelial carcinoma and soft tissue sarcoma (Jimenez *et al.*, 2000; Brandwein-Gensler *et al.*, 2005; Carneiro *et al.*, 2011; Tsukushi *et al.*, 2014). Of invasive patterns in veterinary malignancies, vascular invasion has received the most attention as a possible prognostic indicator (Simko *et al.*, 2003; Barrera *et al.*, 2013; Campos *et al.*, 2014; Diessler *et al.*, 2017). An improved understanding of the leading edge of these tumours might also influence surgical planning and histological evaluation of excisional status (Reimer *et al.*, 2005; Schultheiss *et al.*, 2011; Upchurch *et al.*, 2014; Risselada *et al.*, 2015, 2016a,b; Reagan *et al.*, 2016; Bray, 2017; Milovancev and Russell, 2017).

The clinical relevance of tumoural invasion in MCT and STS is met with inconsistent data in the veterinary literature. In canine STS the significance of invasion is conflicting (Postorino *et al.*, 1988; Baker-Gabb *et al.*, 2003; Stefanello *et al.*, 2008; Chase *et al.*, 2009). This may in part reflect inherent limitations of using in-vivo tumour mobility as an estimate of tumour invasion (Stefanello *et al.*, 2008; Chase *et al.*, 2009). Recently, categorical classification of tumour depth and growth pattern has been suggested as potentially clinically relevant (Avallone *et al.*, 2014). In subcutaneous canine MCTs, growth pattern may have prognostic significance, although by definition this cohort excluded tumours that invaded the dermis (Thompson *et al.*, 2011). The importance of invasion in cutaneous MCTs is also unclear. One study using a subjective evaluation of invasion in cutaneous MCTs found no association with outcomes (Schultheiss *et al.*, 2011). Another study of cutaneous MCTs found no prognostic association with tumour depth, although the statistical model did not take into account tumour grade, growth patterns such as circumferential or vascular invasion, or excisional status (Kiupel *et al.*, 2005). In order to determine the relevance of specific histomorphological invasive patterns, they need to be clearly and objectively defined, quantified and compared against known clinically relevant indices. Studies might also consider the likely possibility that detection of both invasive growth patterns and margin status are influenced by sectioning technique (Dores *et al.*, 2017; Milovancev and Russell, 2017).

The primary aim of the present study was to build on existing veterinary literature by defining objective and quantifiable invasive growth patterns of the leading edge in low-grade canine MCTs and grades I and II STSs. To lend clinical relevance to these findings, we investigated the relationship between invasive growth patterns, clinical animal variables and completeness of excision. We hypothesized that there would be significant differences in quantified invasive growth patterns between MCTs and STSs. A secondary aim was to evaluate the relationship between invasive growth patterns and completeness of excision. The results presented herein enhance understanding of the leading edge of MCTs and STSs, as well as providing insight into objective measures of tumour growth at the leading edge.

Materials and Methods

Samples of MCT and STS were taken from client-owned dogs enrolled in a previously published, institutional animal care and use committee-approved, prospective clinical study with independent research objectives and hypotheses (Milovancev *et al.*, 2017). Animal clinical data collected for this study included signalment, body weight, body condition score (9-point scale), clinical stage including maximum tumour diameter (measured preoperatively using a caliper), intraoperative in-vivo grossly normal circumferential surgical margin lengths in four directions for each tumour (e.g. cranial, dorsal, caudal and ventral; measured using a sterile caliper prior to incision) and surgeon-reported number of fascial planes resected *en bloc* with each tumour. All surgeries were performed, or directly supervised by one of two board-certified veterinary small animal surgeons (MM or KLT). Surgical margins were inked as previously described (Milovancev *et al.*, 2017).

Following complete fixation in 10% neutral buffered formalin, all excisional samples were sectioned identically by one board-certified veterinary anatomical pathologist (DSR). Tissues were cut radially, in four perpendicular directions representing the five margins of interest (i.e. cranial, caudal, lateral, medial and deep). Each radial section aimed to capture both the palpable tumour, invasive front, histologically normal tissue and the inked surgical edge. Sections >2.5 cm long and >2 cm wide were subsectioned to facilitate tissues fitting into the cassette, as recommended by an American College of Veterinary Pathologists consensus committee (Kamstock *et al.*, 2011). Tissues were processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE) according to standard operating procedures at the Oregon Veterinary Diagnostic Laboratory.

A single board-certified veterinary anatomical pathologist (DSR) evaluated all slides using an Olympus BX46 microscope with measurements