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Malignant canine mammary tumours: Preliminary genomic insights using oligonucleotide array comparative genomic hybridisation analysis

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ABSTRACT

Neoplastic mammary disease in female dogs represents a major health concern for dog owners and veterinarians, but the genomic basis of the disease is poorly understood. In this study, we performed high resolution oligonucleotide array comparative genomic hybridisation (oaCGH) to assess genome wide DNA copy number changes in 10 malignant canine mammary tumours from seven female dogs, including multiple tumours collected at one time from each of three female dogs. In all but two tumours, genomic imbalances were detected, with losses being more common than gains. Canine chromosomes 9, 22, 26, 27, 34 and X were most frequently affected. Dissimilar oaCGH ratio profiles were observed in multiple tumours from the same dogs, providing preliminary evidence for probable independent pathogenesis. Analysis of adjacent samples of one tumour revealed regional differences in the number of genomic imbalances, suggesting heterogeneity within tumours.

Malignant canine mammary tumours (MCMTs) manifest as a highly heterogeneous disease, appearing frequently as multiple nodules (Sorenmo et al., 2011). In human oncology, determining the relationship between multiple breast tumours is mandatory for establishing therapeutic strategies (Bendifallah et al., 2010). Genomic testing is a powerful tool for the investigation of genome organisation in individual tumours, and to assess the relatedness of multiple tumours from one patient (Teixeira et al., 2004).

Recently, only three studies have described genomic aberrations in MCMTs (Beck et al., 2013; Liu et al., 2014; Borge et al., 2015). A high-resolution canine oligonucleotide array CGH (oaCGH) platform is now available, offering valuable opportunities to identify genome-wide DNA copy number aberrations (CNAs) associated with carcinogenesis (Breen and Thomas, 2012; Poorman et al., 2015).

The aim of this study was to identify DNA CNAs in MCMTs of different histological types using oaCGH. In order to detect different clonal populations within tumours, paired samples from separate parts of each tumour were collected from two MCMTs. Moreover, multiple tumours collected at one time from a single dog were analysed to evaluate clonal relatedness.

Ten spontaneously arising MCMTs were obtained from seven female dogs that underwent surgery. From three female dogs, samples from two macroscopically independent MCMTs (designated as T*number*) were analysed. Sample acquisition was performed under an institutionally approved protocol (Approval number 17/2012, 30 April 2013). Tumours were evaluated by histopathology to establish classification and grade (Elston and Ellis, 1991; Goldschmidt et al., 2011). Immunostaining was performed to confirm the tumour cell types (Appendix: Supplementary Table S1). In this series, four complex carcinomas (CC), three simple carcinomas (SC), two carcinosarcomas (CS) and one mixed-type carcinoma (MC) were included (Appendix: Supplementary Table S2). Actin- and p-63-positive proliferating myoepithelial cells were observed in all the CCs and CSs, and in the single MC.

Frozen tissue samples representing each histologically evaluated case were used as a source of DNA, which was isolated using routine protocols. Each specimen was then assessed for the presence of genome wide DNA copy changes using data derived from a 180,000 feature canine microarray (design 025522, Agilent Technologies), as previously described (Poorman et al., 2015).

Genomic imbalances were detected in all but two tumours (Table 1), with an average of three detectable alterations per sample (range, 0–11). CNAs were distributed throughout the karyotype and usually involved more than one chromosome. Most of the CNAs detected were in the form of whole chromosome aneuploidy, with a

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Table 1
Histopathological features and major oaCGH copy number changes (CNAs) of 10 canine mammary malignant tumours.

Dog number	Tumour	Diagnosis	Grade	Cell types/other elements	CNAs
1	T1	CC	II	E, npME/squamous cells, fibrovascular stroma	Highly complex 26 + del of 11, 19 and 22 and gain of 24 in <25% of the cells.
2	T1	CC	II	E, npME/fibrovascular stroma	Sample a: Gain of 4, 9 and 13 + loss of 12 and 27 in <25% of the cells. Sample b: Gain of 4, 9 and 13 + loss of 12 and 27 in <25% of the cells.
	T2	SC	II	E/lipid-rich cells	Broad baseline[a], aberrations in 4, 9, 13
3	T1	CC	II	E, npME/squamous cells, fibrovascular stroma	Del 22, gain 34 (with del)
4	T1	CC	I	E, npME/squamous cells, fibrovascular stroma, inflammatory cells	Del 22, gain 34 (with del)
	T2	CS	II	E, npME, mM/immature cartilage and bone, squamous cells, inflammatory cells	Loss on Xp
5	T1	MC	II	E, npME, nM/mature bone and cartilage	No major CNAs
	T2	SC	II	E/scirrhous stroma	No major CNAs
6	T1	CS	II	E, npME, mM/immature cartilage, scirrhous stroma	Sample a: Loss 3, loss 5, del 6 dist, gain 9 (with del), gain 12 dist, loss 15, gain 17, loss 18, gain 24, loss 27, gain on Xp Sample b: Gain on Xp
7	T1	SC	II	E/squamous and lipid-rich cells	Broad baseline[a], gain 9, complex 15, 18, 23 and 26

CC, complex carcinoma; CS, carcinosarcoma; del, deletion; E, epithelial; MC, mixed-type carcinoma; ME, myoepithelial; M, mesenchymal; m, malignant; n, non-malignant; p, proliferating; SC, simple carcinoma; T, tumour.
[a] Poor DNA sample.

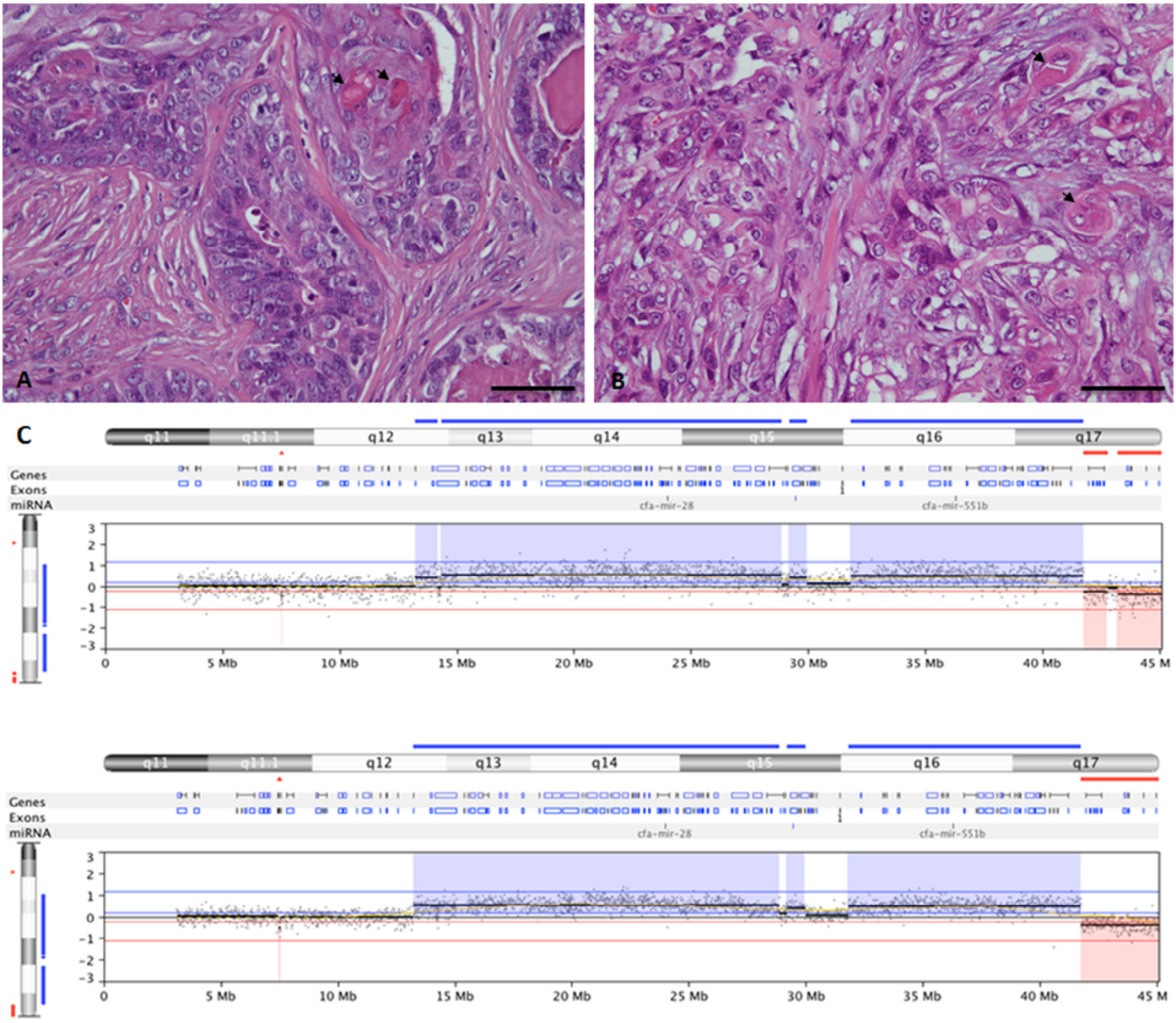

Fig. 1. Complex carcinomas from two female dogs (A, T1 case 3; B, T1 case 4). The tumours shared genome wide similarity in copy number aberrations, and some phenotypic features, such as squamous metaplasia (arrows). (C) Oligonucleotide array comparative genomic hybridisation (oaCGH) profile along the length of canine chromosome (CFA) 34 in both tumours showing an almost identical DNA copy number profile. Haematoxylin–eosin; Bar: 50 μm (A and B).