GENETIC ALTERATIONS IN FOUR CASES OF CANINE MAMMARY TUMOURS: TWO TRISOMIES, TRANSLOCATIONS AND ONE TUMOUR SUPPRESSOR GENE p53 MUTATION

ALTERACIONES GENÉTICAS EN CUATRO CASOS DE TUMORES MAMARIOS CANINOS: DOS TRISOMÍAS, TRANSLOCACIONES Y UNA MUTACIÓN EN EL GEN SUPRESOR TUMORAL p53

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Palabras clave adicionales

Anomalías cromosómicas. Tumores caninos.

SUMMARY

Mammary tumours of different types of ten dogs were subjected to cytogenetic analyses. In one of the patients (a 9 years old boxer-breed) we detected trisomy 1 (2n= 79) in 5 of the 40 (12.5 p. cent) investigated cells. This tumour was a benign mammary mixed tumour showing a strong chondroid component. In a second patient (a 13 years old boxer mixed breed) we observed trisomy 9 (2n= 79) in 4 of the 50 (8.0 p. cent) screened cells of this papillary adenocarcinoma of the complex type. In a third patient (a 7 years old poodle) we observed a reciprocal translocation t (1;19) and 2n= 78 in 4 of the 25 (16 p. cent) investigated cells. Another 3 cells (12 p. cent) of this papillary adenocarcinoma of the complex type showed centric fusion 11/16 (2n= 77). Moreover, the same 10 tumours were subjected to a gene sequencing examination of exons 1 and 2 of the proto-oncogenes K-ras, N-ras and H-ras and exons 5, 6, 7 and 8 of tumour suppressor gene p53. The only mutation present was a GGC→GCC (glycine→alanine) mutation at position 245 of exon 7 (according to human codon numbering) in a complex type mammary carcinoma of a 12 years old German shepherd mixed breed.

RESUMEN

Tumores mamarios de diferentes tipos procedentes de 10 perros fueron sometidos a análisis citogenético. En uno de los pacientes (un ejemplar boxer de 9 años de edad), detectamos trisomía 1 (2n= 79) en 5 de las 40 células investigadas (12,5 p. cien del total). En este caso se trataba de un tumor mamario mixto benigno que mostraba un fuerte componente condroide. En un segundo caso (un ejemplar de 13 años cruzado con boxer), observamos trisomía 9 (2n= 79) en 4 de las 50 células analizadas (8,0 p. cien) en el adenocarcinoma papilar de tipo complejo que presentaba. En un tercer caso (un caniche de 7 años de edad) observamos una translocación recí-

proca t (1;19) y 2n= 78 en 4 de las 25 células investigadas (16 p. cien del total). Otras tres células de este adenocarcinoma papilar de tipo complejo mostraron fusión céntrica 11/16 (2n= 77). Además, los mismos 10 tumores fueron sometidos a un examen de las secuencias génicas de los exones 1 y 2 de los protooncogenes K-ras, N-ras y H-ras y los exones 5, 6, 7 y 8 del gen supresor tumoral p53. La única mutación presente fue una mutación del tipo GGC→GCC (glicina→alanina) en la posición 245 del exón 7 (siguiendo la numeración de codones humanos) en un carcinoma mamario de tipo complejo procedente de un ejemplar cruzado de pastor alemán de 12 años de edad.

INTRODUCTION

Very little is known about the tumour cytogenetics of domestic animals. There is particular need for information about the characterization of clinically important tumours, including their chromosome banding, and in the case of canine tumours there are some remaining difficulties with the karyotype of this species. Because of the importance of different mammary tumours in daily canine veterinary patients, we targeted these diseases for cytogenetic and molecular genetic analyses.

Molecular genetic studies on oncogenes and tumour suppressor genes are extremely rare in canine mammary tumours. Therefore, we studied our 10 patients also for mutations in *Tumour hotspot* regions of three ras genes (K-, N, H-) and the p53 antioncogene.

MATERIAL AND METHODS

Tumour material from ten mammary tumour bearing dogs was analyzed cytogenetically. Additionaly, molecular genetic studies were performed in the same dogs involving genomic K-ras, N-ras, H-ras oncogenes and p53 tumour suppressor gene.

For cytogenetic studies, primary explant cell cultures were established by mincing the solid tissue into small fragments (less than 1 mm). The fragments were transferred into sterile flasks containing 8 ml RPMI 1640 medium with L-glutamine, antibiotics (50 iu penicillin and 50 μg streptomycin ml^{-1}) and 10 per cent fetal calf serum (all from Gibco). The explants were cultured in 5 per cent carbon dioxide in air for 10 days until harvesting. The metaphases were analyzed by G-banding by the method of Wang and Fedoroff (1972).

For molecular genetic ras and p53 analyses, DNA was extracted from tissue samples of the 10 tumours in accordance with standard techniques (Müllenbach *et al.*, 1989). Polymerase chain reaction (PCR) primers for the amplification of the N-, K- and H-ras fragments were designed on the basis of previously published human and mammalian sequencing data. The specific oligonucleotides and the PCR conditions used to generate N-ras exon I and exon II fragments have been described previously (Watzinger *et al.*, 1994). Synthetic oligonucleotide primers used in amplification of K- and H-ras sequences were as follows: K-ras Ia S, 5'gAC TgA ATA TAA ACT TgT gg-3', and K-ras Ia AS, 5'-CTA TTg TTg gAT CAT ATT Cg-3' generating a 107 bp fragment from exon I; K-ras IIa S, 5'-ATT CCT ACA ggA AgC AAg-3', and K-ras IIa AS, 5'-CTA TAA Tgg TgA ATA TCT TC-3' generating a 178 bp fragment from exon

II; H-ras Ia S, 5'-gAC ggA ATA TAA TCT ggT-3', and H-ras Ia AS, 5'-TCg ATg gTg ggg TCg TAC TC-3' generating a 108 bp fragment from exon I; H-ras IIa S, 5'-gAC TCC TAT Cgg AAg CAA gT-3', and H-ras IIa AS, 5'CCT gTA CTg gTg gAT gTC C-3' generating a 181 bp fragment from exon II. Conditions of amplification were as described earlier (Watzinger *et al.*, 1994), the only modification being an adaptation of the annealing temperature for K-ras exons I and II and H-ras exon I to 50°C, and for H-ras exon II to 60°C.

The primers and the PCR conditions used to generate P53 exon 5, exon 6, exon 7 and exon 8 fragments have been described earlier (Kraegel *et al.*, 1995). The PCR products were analysed by 4 p. cent NuSieve/Agarose gel electrophoresis. The products were extracted from the TBE gels, following the Geneclean II Kit (Bio 101 Inc., La Jolla, California, US) procedure. The PCR products were sequenced using the Taq Dye Deoxy Terminator Cycle Sequencing Kit and an Automatic sequencer, ABI 373 A (Applied Biosystems, Foster City, California, US). The four distinctly fluorescent-labelled dideoxynucleotides were used for chain termination in the PCR. Each of the four dyes fluoresced at a different wavelength. During 7 p. cent polyacrylamide gel migration, the fluorescent-labelled DNA fragments were excited by an argon laser at a fixed position. Detectors registered the fluorescence dye-specific signal and gave the user the analysed sequencing data in the form of four-colour chromatograms. All sequences were obtained for both strands. The DNA was amplified and sequenced three times in order to exclude PCR artefacts.

RESULTS

The parallel study of 10 mammary tumours of ten canine patients for chromosomal alterations and mutations in the N-, K- and H-ras-oncogenes and the p53-tumour-suppressor gene revealed alterations in 4 cases. In a benign mammary mixed tumour with a pronounced tendency to chondroid metaplasia of a 9 years old boxer there was a trisomy 2n=79 **(figure 1)** in 5 of the 40 (12.5 p. cent) analysed cells. In the papillary adenocarcinoma of the complex type 13 years old boxer-mixed breed we detected trisomy 9 and 2n=79 **(figure 2)** in 4 of the 50 (8 p. cent) analysed cells.

In a further papillary adenocarcinoma of the complex type in a 7 years old poodle we observed a reciprocal translocation t(1;19) and 2n=78 **(figure 3)** in 4 of the 25 (16 p. cent) screened cells.

The studies in exons 5, 6, 7 and partial 8 of tumour suppressor gene p53 revealed only one mutation. In a 12 years old German shephard dog we found a transversion GGC → GCC (glycine→ alanine) in codon 245 in exon 7 **(figure 4)**.

The studies in exons 1 and 2 of K-, N- and H-ras-oncogenes did not reveal a mutation in any of the ten tumours.

DISCUSSION

In the present study, trisomy 1 was found in a benign mammary mixed tumour showing a strong chondroid component. Similarly, trisomy 1 were reported in a malign mammary mixed tumour with metaplastic ossification (Mayr *et al.*, 1991) and in a complex type mammary adenocarcinoma (Mayr *et al.*,

1993). Together with trisomy 9 in a papillary adenocarcinoma, complex type in our present study and several trisomies (trisomy 2, 5, 6 and 10) reported by Reinmann *et al.* (1996) they provide excellent candidates for the potential importance of trisomies and unbalanced karyotypes in the initiation and particularly in the progression of canine mammary tumours.

Our finding of the reciprocal translocation t(l/19) in a papillary

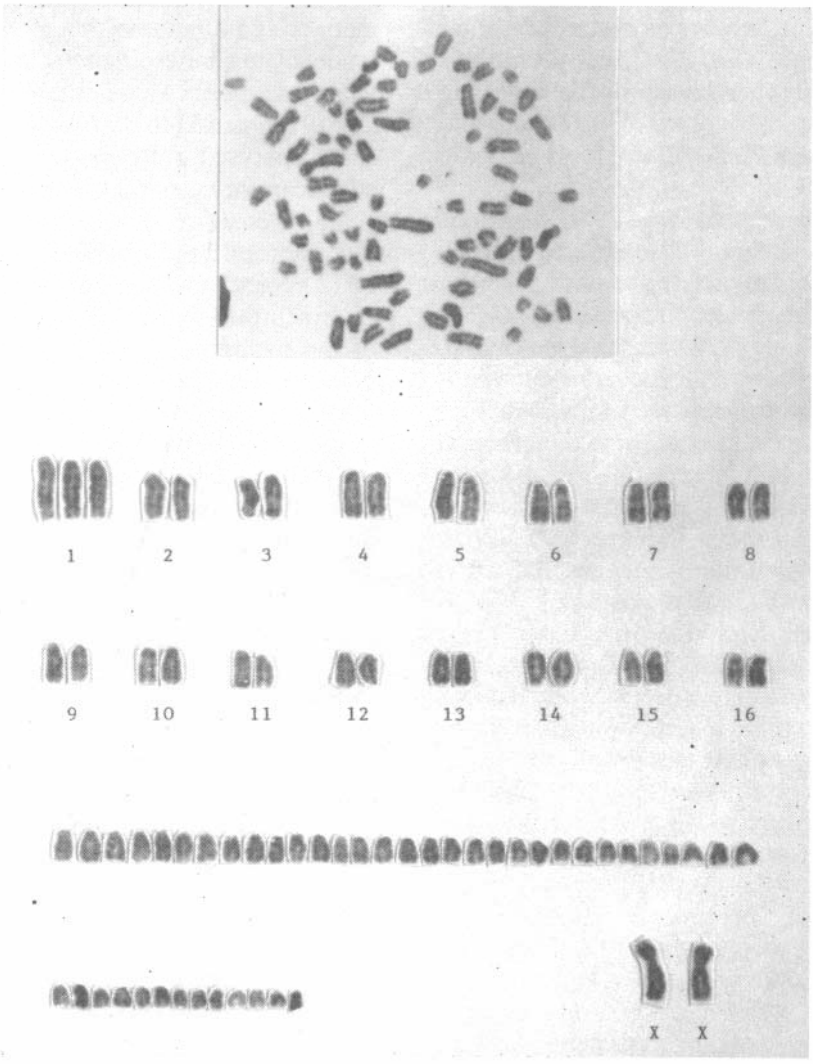

Figure 1. *Metaphase and karyotype of a benign mammary mixed tumour. Note trisomy 1. 2n= 79.*
(Metafase y cariotipo de un tumor mamario benigno mixto. Obsérvese la trisomía 1. 2n= 79).

adenocarcinoma is hard to evaluate at present because of the paucity of literature on reciprocal translocations in canine mammary tumours. A report about a reciprocal translocation t(4/27) in a mammary complex adenocarcinoma (Mayr *et al.*, 1990) is just one exception from this striking lack of data.

Exons 1 and 2 of the three investigated ras genes and exons 5, 6, 7 and 8 of the p53 gene are wellknown for harbouring tumour *hot spots* in human oncology.

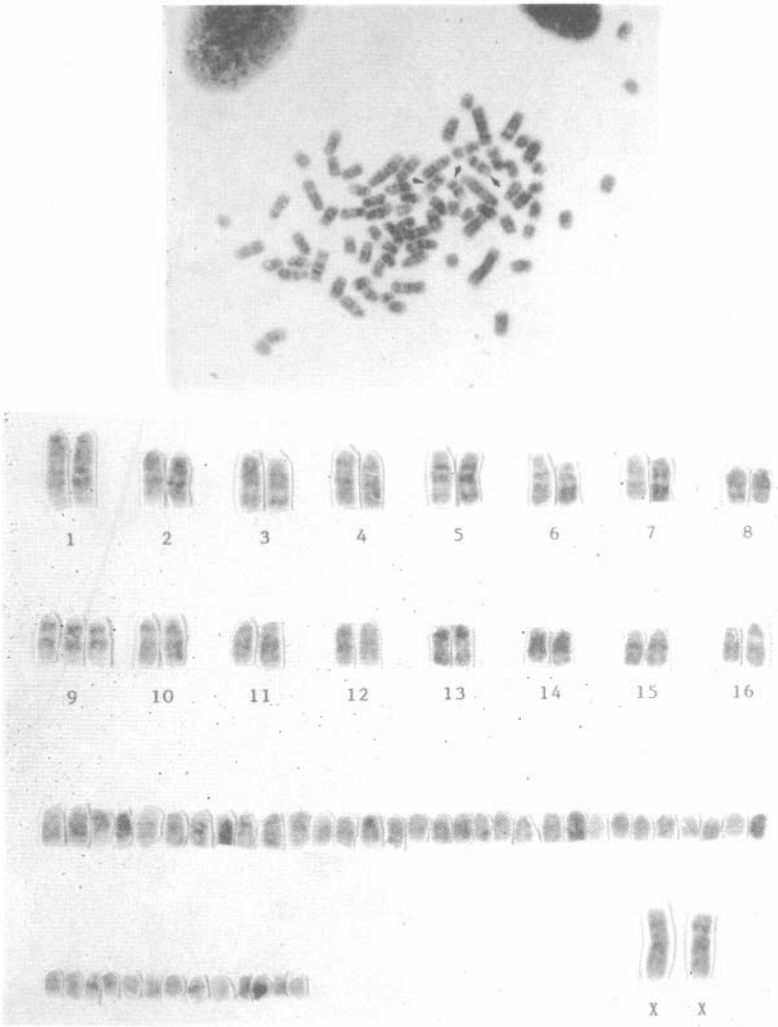

Figure 2. *Metaphase and karyotype of a papillary adenocarcinoma of the complex type. Note trisomy 9. 2n=79.* (Metafase y cariotipo de un adenocarcinoma papilar de tipo complejo. Obsérvese la trisomía. 2n = 79).

However no mutations in the ras-genes and only one mutation in exon 7 (codon 273) in p53 were detected in the mammary tumours of our 10 analysed patients. This fact seems to suggest a limited frequency of mutations in these exons in canine mammary tumours. This finding is in good correspondence with N-ras, K-ras and H-ras studies in the domestic cat, where mutations in exons 1 and 2 were also extremely rare in mammary tumours (Mayr *et al.*, 1996, unpublished).

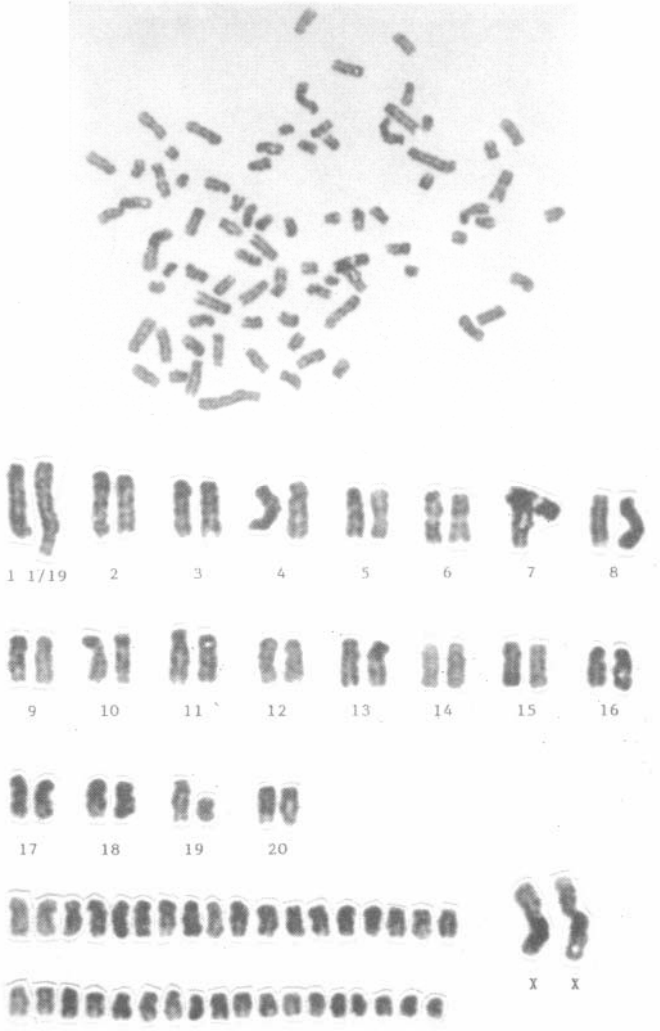

Figure 3. *Metaphase and karyotype of a papillary adenocarcinoma of the complex type. Note reciprocal translocation t(1;19). 2n=78.* (Metafase y cariotipo de un adenocarcinoma papilar de tipo complejo. Obsérvese la transformación recíproca t(1;19). 2n = 78).

225	226	227	228	229	230	231	232	233	234
GTT	GGC	TTT	GAC	TAT	ACC	ACC	ATC	CAC	TAC
V	G	S	D	Y	T	T	I	H	Y
235	236	237	238	239	240	241	242	243	244
AAC	TAC	ATG	TGT	AAC	AGT	TCC	TGC	ATG	GGA
N	Y	M	C	N	S	S	C	M	G
245	246	247	248	249	250	251	252	253	254
GGC/GCC	ATG	AAC	CGG	CGG	CCC	ATC	CTC	ACT	ATC
G / A	M	N	R	R	P	I	L	T	I
255	256	257	258	259	260				
ATC	ACC	CTG	GAA	GAC	TCC				
I	T	L	E	D	S				

Figure 4. *Sequence of canine exon 7. Note the transversion G → C in codon 245 in a complex type mammary carcinoma, leading to amino acid substitution glycine → alanine. The numbering was done in accordance with the human system.* (Secuencia del exon canino 7. Obsérvese la transversión G → C en el codon 245 en un carcinoma mamario de tipo complejo, conducente a la sustitución de aminoácidos glicina → alanina. La numeración se realiza de acuerdo con el sistema humano).

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