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CANINE TUMOUR SUPPRESSOR GENE p53 MUTATION IN A CASE OF ANAPLASTIC CARCINOMA OF THE INTESTINE

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Tumours localised in the large bowel of dogs were subjected to molecular genetic studies. Highly conserved regions of the tumour suppressor gene p53, including typical tumour hot spots (codons 175, 245, 248, 249, 273 and 282), were analysed. A mutation CGG → TGG (arginine → tryptophan) was present in codon 249 in an anaplastic carcinoma in the caecum.

Key words: Carcinoma, caecum, dog, intestine, mutation, p53

In man, colorectal cancer is the second commonest cancer in the developed countries, but it is much rarer in most developing countries. Ideally, the cancers of large bowel like caecal, colonic and rectal cancer should be classified separately, e.g. because of epidemiological differences, but in many cases data for the separate sites are combined (Wasan and Bodmer, 1997). Intestinal tumours in the dog are relatively rare: they account for 0.5 percent of all malignant tumours. The majority of canine large bowel neoplasms are localised in the rectum and the distal third of the colon, while caecal localisation is rare.

The tumour suppressor gene p53 is mutated in a high fraction of human neoplasms including colorectal cancers (Levine et al., 1991; Beroud and Soussi, 1998; Soussi et al., 2000). A high proportion of these alterations affects several hot spots (codons 175, 245, 248, 249, 273 and 282) of the gene in man.

In dogs, p53 alterations have been detected in cases of mammary tumours (Van Leeuwen et al., 1996; Chu et al., 1998; Mayr et al., 1998; Mayr et al., 1999; Veldhoen et al., 1999; Muto et al., 2000), lymphoma (Veldhoen et al., 1998; Nasir and Argyle, 1999; Setoguchi et al., 2001), osteosarcoma (Van Leeuwen et al., 1997; Johnson et al., 1998; Setoguchi et al., 2001), thyroid tumour (Devilee et al., 1994; Setoguchi et al., 2001), papilloma (Mayr et al., 1994), skin tumours (Mayr et al., 1999), colon cancer (Setoguchi et al., 2001) and circumanal gland tumour (Mayr et al., 1997). However, apart from the above-mentioned colon cancer (Setoguchi et al., 2001) virtually no data are available regarding neoplasms of the small and large intestine. Here, we report the finding of a p53 mutation in a caecal carcinoma in a dog.

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Materials and methods

DNA was extracted from tumour specimens and peripheral blood immediately after surgery according to a standard technique (Müllenbach et al., 1989). The 10 randomly collected neoplasms were 6 adenomas and 4 carcinomas of the large bowel. The genomic regions analysed, primers, PCR technique, sequencing technique used and codon numbering (human system) were exactly the same as described earlier (Mayr et al., 1997). It is important to emphasise that not the entire p53 sequence was analysed. Segments of the canine p53 gene covering regions from exons 5 to 8 were amplified separately. The first segment corresponded to part of exon 5 (codons 132–186) including intron 5 (81 bp) and part of exon 6 (codons 187–200) of the gene (Soussi et al., 1990). A 20 bp sense (5′-AAGATGTTTTGCCAACTGGC-3′) and a 17 bp antisense primer (5′-TTTCCTTCCACTCGGAT-3′) were used. The second amplified segment corresponded to exon 7 (codons 277–261), including intron 7 and exon 8 (codons 262–306). A 20 bp sense primer (5′-GTTGGCTCTGACTGTACCAC-3′) and a 19 bp antisense primer (5′-TTACCTCGCTTACTGCTCC-3′) were used for this second amplification.

The PCR buffer was composed of 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.0; 1.5 mmol/L $MgCl_2$ and 0.1% (v/v) Triton X-100. Ampli-Taq-DNA polymerase (Perkin-Elmer Catus, CA, USA) was the enzyme used and 35 amplification cycles were performed. Each cycle consisted of template denaturation (2 min at 97 °C), primer annealing (1 min at 53 °C) and extension (1 min at 73 °C).

The amplification products were eluted from the TBE gel using the Geneclean II Kit (Bio 101 Inc., La Jolla, CA, USA) and sequenced using the Taq Dye Deoxy Terminator Cycle Sequencing Kit and the Automatic sequencer ABI 373 A (Applied Biosystems, Foster City, CA, USA).

Results

In one of the 10 investigated tumours of the large bowel a p53 mutation was detected. In a solid anaplastic carcinoma (Fig. 1) of the caecum of an 8-year-old Cocker spaniel a transition CGG → TGG in codon 249 of exon 7 was observed. This codon alteration results in an amino acid change from arginine to tryptophan. Both the mutated allele TGG and the wild-type allele CGG were detected in the tumour. However, this transition was restricted to the tumour and remained undetected in control blood lymphocytes, thus implying its somatic nature.

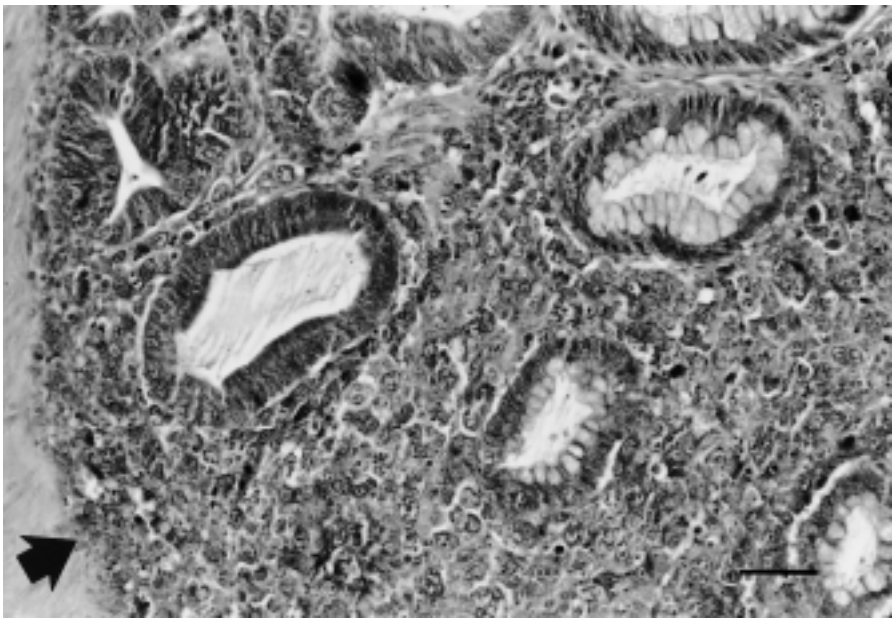

Fig. 1. Solid anaplastic carcinoma of the caecum of a dog; undifferentiated epithelial cells infiltrating the propria of the caecal mucosa; epithelium of some crypts abnormally differentiated; tumour bordering on the muscularis mucosae (arrow). Bar represents 60 μm

Discussion

In the present study a p53 alteration was detected in a neoplasm of the caecum. Histologically, the tumour presented as a solid anaplastic carcinoma with undifferentiated cells infiltrating the mucosa. In man, investigations at various genetic stages of colorectal carcinomas showed that p53 mutations are generally a late event often accompanied by loss of heterozygosity. Thus, the finding of the dedifferentiated nature of our p53 altered neoplasm and the lack of p53 changes in several of our less malignant tumours, e.g. adenomas, are well compatible. The presence of both the mutated and the wild-type alleles of p53 in our tumour may possibly represent the presence of contaminating stromal, inflammatory or adjacent cells but can also be indicators for the genetic heterogeneity of the neoplastic cells themselves. The use of microdissection should reduce this problem in the future.

The p53 change in our anaplastic caecal carcinoma represented a C → T transition at position 249. It seems worthwhile to mention that position 249 has undergone an evolutionary change between amino acid codons AGG in man versus CGG in the dog (the same amino acid arginine in both cases) creating a CpG dinucleotide difference. In man, 80% of the mutations in colorectal cancers (for

comparison, see Soussi et al., 2000) are G → A transitions, which are predominantly located at the CpG dinucleotide, thus implicating deamination events of 5-methylcytosine. On the other hand, G → T transversions are very rare in man and position 249 shows a very low mutation frequency (1%) in comparison to the CpG dinucleotide harbouring hot spots 175, 248 and 273. Remarkably, several canine position 249 point mutations reported so far are CGG → TGG transitions (arginine → tryptophan). They concerned two mammary (Mayr et al., 1998; Muto et al., 2000) and one circumanal gland tumour (Mayr et al., 1997).

In a colon cancer two aberrant p53 alleles with differing mutations were detected; they showed four missense point mutations, one insertion and two deletions (Setoguchi et al., 2001). The use of p53 complementary DNA (cDNA) covering the whole transcription unit including exons 1 to 11 in their study allowed the demonstration of the severity of the damage of both p53 alleles in their colon sample. Future genetic characterisation of various neoplasms of the canine large intestine using p53, other tumour suppressor genes and oncogenes will be worthwhile for a better understanding of canine and comparative oncology.

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