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TUMOUR SUPPRESSOR GENE p53 MUTATION IN A CASE OF HAEMANGIOSARCOMA OF A DOG

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Haemangiosarcomas of dogs were analysed by molecular genetic techniques. Regions of the tumour suppressor gene p53, including the well-known tumour hot spots (codons 175, 245, 248, 249, 273 and 282) were screened. A 24 bp deletion was detected in exon 5 of the gene.

Key words: Haemangiosarcoma, spleen, dog, mutation, p53

Haemangiosarcoma is a malignant vascular tumour which develops from the endothelium of blood vessels. The degree of endothelial differentiation is quite variable and ranges from neoplasms with large, well-defined vascular lumina to tumours showing minimal vascular differentiation, which require careful evaluation to distinguish them from other sarcomas. Primary tumours are most likely localised in spleen, liver, heart, bone marrow and skin of domestic animals and man. The German shepherd is a breed in dogs particularly prone to these tumours (Dahme, 1999).

The tumour suppressor p53 is mutated in a high fraction of human neoplasms including soft-tissue sarcomas (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. There are a few reports of high incidences of p53 mutations in human haemangiosarcomas (Hollstein et al., 1994; Soini et al., 1995; Naka et al., 1997; Marion, 1998; Marion and Boivin-Angele, 1999; Tudek et al., 1999; Amo et al., 2000; Boivin-Angele et al., 2000). In part, these p53 mutations were associated with occupational exposure to chemicals, e.g. vinyl chloride.

In dog p53, very few studies of neoplasms of soft-tissue sarcomas including haemangiosarcomas have been performed (Nasir et al., 2001). Here, we report the finding of a p53 mutation in a haemangiosarcoma in the spleen of 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 15 randomly collected neoplasms were haemangiosarcomas (6, 5, 2 and 2 samples from spleen, liver, heart and skin, respectively). The genomic regions analysed, primers, PCR technique, sequencing technique used and codon numbering (human system) were the same as described earlier (Mayr et al., 1997; Mayr and Reifinger, 2002). 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 p53 gene (Soussi et al., 1990). A 20-nucleotide (nt) long sense (5′-AAGATGTTTTGCCAACTGGC-3′) and a 17-nt antisense primer (5′-TTTCCTTCCACTCGGAT-3′) were used. The second amplified segment corresponded to exon 7 (codons 227–261), including intron 7 and exon 8 (codons 262–306). A 20-nt sense primer (5′-GTTGGCTCTGACTGTACCAC-3′) and a 19-nt antisense primer (5′-TTACCTCGCTTACTGCTCC-3′) were used for this second amplification.

The PCR buffer was composed of 50 mM KCl, 10 mM Tris-HCl, pH 8.0; 1.5 mM $MgCl_2$ and 0.1% (v/v) Triton X-100. Ampli-Taq DNA polymerase (Perkin-Elmer Cetus, CA, USA) was 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).

After electrophoresis, the amplification products were eluted from the TBE gel using Geneclean II Kit (Bio101 Inc., La Jolla, CA, USA) and sequenced using Taq Dye Deoxy Terminator Cycle Sequencing Kit and an automatic sequencer ABI 373 A (Applied Biosystems, Foster City, CA, USA).

Results

From the 15 investigated haemangiosarcoma samples, one p53 mutation was detected in the spleen of an 8-year-old male Setter mixed-breed dog (Fig. 1). A 24-bp deletion encompassing codons 159 to 167 was found, covering the following genome fragment: TT (second and third base of codon 159) - GTG-CGG-CGC-TGC-CCC-CAC-CAT-G (first base of codon 167). Both the deleted and the wild-type allele were present in the tumour. The deletion was restricted to the tumour, but was not observed in control blood lymphocytes. Thus, the somatic nature of the tumour is evident.

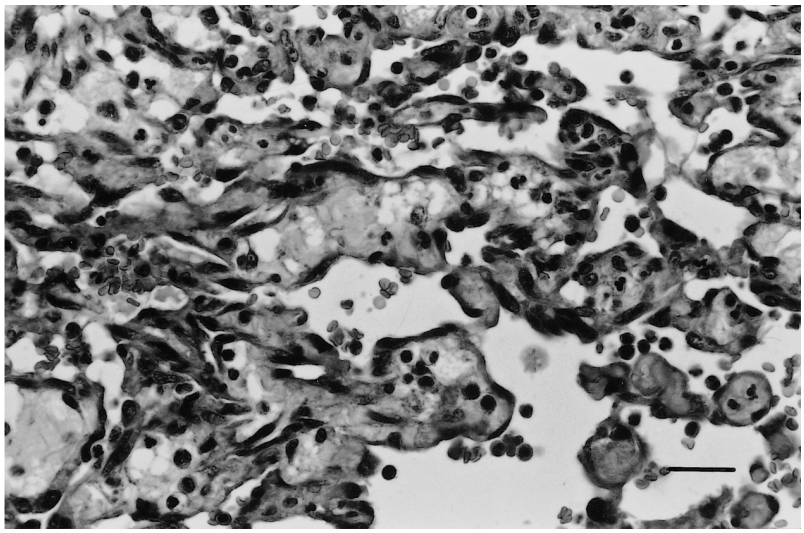

Fig. 1. Splenic haemangiosarcoma; disordered vascular spaces lined by irregular endothelial cells with hyperchromatic nuclei. Bar represents 30 μm

Discussion

A 24-bp deletion from codon 159 to codon 167 was identified in a haemangiosarcoma of a dog. This deletion will clearly give rise to a defective p53 protein. However, no frameshift and premature stops can be expected as mechanisms for the defects caused by this 24-bp deletion. Functionally, it may be important that the deletion is localised within the DNA-binding central domain (codons 102 to 292) of the p53 gene. However, it is not localised within highly conserved regions (regions 2 and 3 are localised from codon 120 to 143 and 172 to 182, respectively).

It is interesting that the mutation detected in our investigation was a deletion. In most of the reported human haemangiosarcomas, the mutation type was point mutation, often G:C→A:T transitions and A:T→T:A transversions (Soini et al., 1995; Naka et al., 1997; Marion, 1998; Marion and Boivin-Angele, 1999; Boivin-Angele et al., 2000). Possibly, factors like the occupational exposure to certain chemicals e.g. vinyl chloride and other exogenous and endogenous factors contribute to the mutation type. In the canine soft-tissue tumours studied (Nasir et al., 2001), G→A transitions and G→T transversions were detected.

In any case, information about mutations in tumour suppressor genes and oncogenes in endothelial cells and their derivative tumours is extremely limited. The somatic p53 mutation of the deletion type in our canine haemangiosarcoma patient could give impetus to the search for different types of p53 mutations e.g. in the context of investigations of different exogenous and endogenous genetic insults in endothelial cells.

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