

N-ras MUTATION IN A CANINE LYMPHOMA: SHORT COMMUNICATION

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Lymphomas of dogs were investigated by molecular genetic methods. Regions of exon 1 and 2 of the N-ras gene, which harbours the mutation hot spots (codons 12, 13 and 61) were screened. A GGT \Rightarrow GAT (glycine \Rightarrow aspartic acid) mutation in codon 13 was present in a multicentric-type lymphoma of a 1-year-old male dog.

Key words: Lymphoma, dog, mutation, N-ras

The N-ras protooncogene is a member of the ras gene family. The extreme sequence similarity within ras exons 1 and 2 in different mammals including several domestic animal species has been demonstrated (Watzinger et al., 1998).

Activation of the N-, K- and H-protooncogenes mostly occurs at codons 12, 13 or 61 in exons 1 and 2 by point mutations. N-ras mutations have often been found in human melanomas, myeloid disorders and thyroid tumours (Bos, 1989; Barbacid, 1990; Rodenhuis, 1992; Teich, 1997; Soussi, 2001).

Quite in contrast to human oncology, almost no N-ras mutations are known in domestic animals. In the domestic dog, N-ras was originally sequenced by Saunders et al. (1992) and the only reported canine N-ras mutation was a transition in codon 13 in a lymphoma (Edwards et al., 1993).

In the present study, we analysed 10 lymphoma-bearing dogs (aged 1 to 8 years) for the presence of N-ras mutations. DNA was extracted from the tumour samples in accordance with standard methods (Müllenbach et al., 1989). For polymerase chain reaction (PCR), we designed primers for parts of exons 1 and 2, including the mutation hot spot codons 12, 13 and 61. The sense primer for exon 1 was 5'-TACAAACTGGTGGTGGTTGGAGC-3', the antisense primer for exon 1 was 5'-CTATGGTGGGATCATATTCATCTAC-3'. The sense primer for exon 2 was 5'-TCTTACCGAAAACAGGTGGTTATAG-3', the antisense primer for exon 2 was 5'-GTCCTCATGTATTGGTCTCTCATGGCAC-3'. PCR, single strand

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conformation polymorphism (SSCP) and DNA-sequencing analyses were performed according to standard procedures.

Details of the conditions used in PCR and DNA sequencing were given in earlier reports (Mayr and Reifinger, 2002; Mayr et al., 2002*b*). For SSCP, 5 µl of the PCR product was mixed with 5 µl of SSCP loading buffer (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, 10 mM EDTA). The DNA samples were heat denatured at 90 °C for 5 min, chilled on ice and applied to a 12.5% polyacrylamide gel (Amersham Pharmacia Biotech AG, Uppsala, Sweden). Electrophoresis was performed in 0.5 × TBE for 150 min at 140 V and 4 °C. Then the gel was subjected to staining by a DNA silver staining kit (Amersham Pharmacia Biotech AG, Uppsala, Sweden).

Only 1 of the 10 investigated patients (10%) showed an alteration in the proven hot spot codons 12, 13 and 61 harbouring regions of codons 1 and 2. This case was a canine lymphoma of multicentric type (lymph node, spleen, gut, thymus, liver, kidneys, bone marrow) in a 1-year-old male patient. Figure 1 shows a typical alteration in the kidney. The SSCP analysis revealed a clear-cut shift (Fig. 2). The mutation sequence was a GGT ⇒ GAT transition (G ⇒ A) giving rise to the amino acid change G ⇒ D (glycine ⇒ aspartic acid) at amino acid position 13.

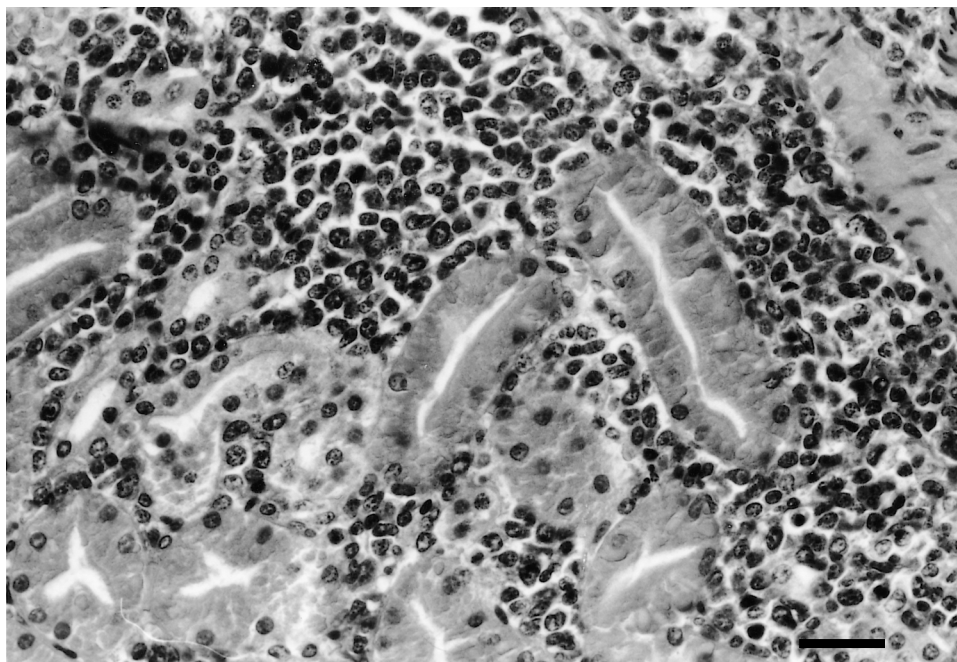


Fig. 1. Canine lymphoma of multicentric type in a 1-year-old male Labrador dog. Diffuse infiltration of the renal interstitium by heterogeneous medium-sized lymphocytes with moderate amount of cytoplasm. Bar represents 30 µm

Intriguingly, our GGT \Rightarrow GAT mutation on codon 13 is totally identical to the mutation reported by Edwards et al. (1993). The 50 analysed cases presented up to now in the literature comprise 28 malignant lymphomas with one N-ras mutation (Edwards et al., 1993) together with 7 (Watzinger et al., 2001) and 15 cases (Mayr et al., 2002a) without N-ras mutations, respectively. Thus, in summary with our present study, the same mutation was detected in 2 of 60 lymphoma patients (3.2%). It is interesting that just the same base substitution GGT \Rightarrow GAT is by far the most frequent N-ras codon 13 mutation in human lymphoid malignancies, too.

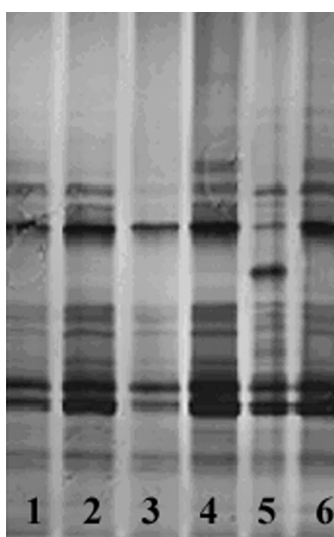


Fig. 2. Single-strand conformation polymorphism analysis (SSCP) of canine N-ras exon 2 of lymphomas of 6 patients. Note the mutation in lane 5

Moreover, the overall low frequency of N-ras mutations in these canine patients is in full accordance with data known from human lymphoid neoplastic diseases (Neri et al., 1988; Browett and Norton, 1989; Ahuja et al., 1990; Nakao et al., 2000). In any case, the present finding is a further contribution to the progress of lymphoma research in veterinary and comparative medicine.

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