Cytogenetic, ras, and p53: Studies in Cases of Canine Neoplasms (Hemangiopericytoma, Mastocytoma, Histiocytoma, Chloroma)

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Four case reports of mesenchymal neoplasms showing chromosomal abnormalities are presented. In a case of hemangiopericytoma trisomy 2 and centric fusion 19;21 were present. In a mastocytoma a deleted chromosome 35 was seen. A homogeneously staining region (HSR) on chromosome 1 was detected in a histiocytoma. Trisomy 5 and monosomy 31 were observed in a case of granulocytic sarcoma (chloroma). The lack of mutations in exons 1 and 2 of oncogenes N-ras, K-ras, and H-ras and exons 5, 6, 7, and 8 of tumor suppressor gene p53 in these four patients and in a larger series of investigated dogs (25 hemangiopericytomas, 12 mastocytomas, and 8 histiocytomas) is highlighted.

Despite the importance of neoplasms in canine patients in veterinary medicine, chromosome banding studies for a better genetic understanding of these diseases are extremely rare. This is due to the problems of difficult identification of canine chromosomes. In the present studies, we give banding reports on chromosome aberrations in tumor cultures from a case each of hemangiopericytoma, mastocytoma, histiocytoma, and granulocytic sarcoma (chloroma). The same four patients were also analyzed for alterations of N-, K-, and H-ras oncogenes in exons 1 and 2 and also for tumor suppressor gene p53 mutations in exons 5, 6, 7, and 8 by PCR/DNA sequencing. The ras and p53 studies were also performed in another group of patients. These regions are well known as tumor “hot spots” in several types of neoplasms in man (Barbacid 1990; Hollstein et al. 1991; Levine et al. 1991; Rodenhuis 1992).

Materials and Methods

A 13-year-old beagle bitch (dog 1) developed a skin tumor in the belly region. The histopathological diagnosis was a mastocytoma (Figure 1). A 5-month-old staffordshire bull terrier (dog 2) bore a skin tumor histopathologically classified as a histiocytoma (Figure 2). A 6-year-old bulterrier (dog 3) developed a skin tumor in the neck region that was classified as a granulocytic sarcoma (chloroma, Figure 3). Another patient, a 13-year-old dachshund bitch (dog 4) had a hemangiopericytoma (Figure 4) in the region of the right front paw. All four dogs were examined in general practices in Vienna. In all four cases, the regional lymph nodes were unaffected and no recurrences were observed after the tumors were removed surgically in November 1996.

Primary explant cell cultures were established by mincing the solid tissue into small fragments (less than 1 mm³). 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) and 10% fetal calf serum (all from Gibco). The explants were cultured in 5% carbon dioxide in air for 2 weeks until harvesting. The fibroblast contamination was very low. Trypsin G-banding (Wang and Federoff 1972) and chromosome nomenclature followed published reports (Reimann et al. 1996; Selden et al. 1975; Switonski et al. 1996). Fifty metaphases were analyzed from each culture.

DNA was extracted from tumor samples in accordance with standard techniques (Müllenbach et al. 1989). The 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 1 and exon 2 fragments have been described previously (Watzinger et al. 1994).

Synthetic oligonucleotide primers used in amplification of K- and H-ras sequences were as follows:
Figures 1–4. (1) Mastocytoma, dog 1. Malignant tumour, composed of oval to polygonal cells with pale nuclei and marked granular cytoplasm; toluidine blue. Bar = 30 μm. (2) Histiocytoma, dog 2. Round to polygonal histiocyte-like cells within the superficial dermis; considerable mitotic activity, few binucleate cells; close contact with the basal epidermal layer (arrows). Bar = 30 μm. (3) Granulocytic sarcoma, dog 3. Extramedullary proliferation of myeloid tumour cells, neutrophil type; cell differentiation varying from low to high. Bar = 45 μm. (4) Hemangiopericytoma, dog 4. Curls and concentric layers of tumorous fibroblast-like cells and collagen fibers around small to medium-size vessels. Bar = 60 μm.

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 1; K-ras Ia S, 5'-ATT CCT ACA ggA AgC AAg-3', and K-ras Ia AS, 5'-CTA TAA Tgg TgA ATA TCT TC-3', generating a 178 bp fragment from exon 2; 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 1; H-ras Ia S, 5'-gAC TCC TAT Cgg AAg CAA gT-3', and H-ras Ia AS, 5'-CCT gTA Ctg gTg gAT gTC C-3', generating a 181 bp fragment from exon 2. 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 1 and 2 and H-ras exon 1 to 50°C, and for H-ras exon 2 to 60°C. The enzyme used was Ampli-Taq DNA polymerase (Perkin-Elmer-Cetus, Foster City, CA).

A segment of tumor suppressor p53 containing exons 5, 6, 7, and 8 was amplified. The primers and PCR-conditions were taken from Kraegel et al. (1995).

The PCR products were analyzed by 4% NuSieve/agarose gel electrophoresis. Amplification resulted in a single discrete band and no nonspecific bands were observed. The products were extracted from the TBE gels, following the Gene cleanup II Kit (Bio 101 Inc., La Jolla, CA) 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, CA). The four distinctly fluorescently labeled dideoxynucleotides were used for chain termination in the PCR. Each of the four dyes fluoresced at a different wavelength.

During 7% polyacrylamide gel migration, the fluorescently-labeled DNA fragments were excited by an argon laser at a fixed position. Detectors registered the fluorescence dye-specific signal and gave the user the analyzed sequencing data in the form of four-color chromatograms. All sequences were obtained for both strands. The DNA was amplified and sequenced three times in order to exclude PCR artifacts. Furthermore, an additional group of 25 hemangiopericytomas, 12 mastocytomas, and 8 histiocytes was molecular genetically screened in the above-mentioned exons of the ras and p53 genes, but no cytogenetic analysis was performed in these patients.

**Results and Discussion**

In the mastocytoma of dog 1, four (8%) of the metaphases had a deleted chromosome 35, giving rise to the karyotype 78,XX,del35 (Figure 5). The histiocytoma of dog 2 showed in 6 (12%) of the metaphases an elongation of the distal region of chromosome 1 (Figure 6). This was due
to a homogeneously staining region (HSR), probably as a result of DNA amplification. The chromosome number was $2n = 78$.

In the granulocytic sarcoma (chloroma) of dog 3 we detected in 5 metaphases (10%) $2n = 78$, a trisomic chromosome 5 and a monosomic chromosome 31. Thus this karyotype was $78,XY,+5,−31$ (Figure 7).

In the hemangiopericytoma of dog 4 we detected in 4 metaphases (8%) $2n = 78$, trisomy 2, and a centric fusion 19:21 (Figure 8). In 10 other metaphases (20%) we found $2n = 78$, trisomy 35, and centric fusion 19:21.

No mutations were detected in exons 1 and 2 of the three analyzed members of the ras oncogene family (N-ras, K-ras, H-ras) in our four both cytogenetically and molecular genetically investigated neoplasms. Analogously, no mutations were detected in exons 5, 6, 7, and 8 of tumor suppressor gene p53 in these four tumors.

Further, in the group of patients which was not studied cytogenetically (25 hemangiopericytoma, 12 mastocytoma, and 8 histiocytoma), no mutations in any of these ras or p53 exons have been found. This result was somewhat surprising to us considering the importance of alterations of these genomic regions in many types of neoplasms in man. More than 3,000 p53 mutations have been cataloged (Cariello et al. 1994; De Vries et al. 1996; Hollstein et al. 1996; Levine et al. 1991). To date it is not known whether the absence of p53 mutations in the tumors analyzed by us is a valid result or whether sequencing of the whole gene rather than a specific sequence (exons 5–8) would reveal mutations. Moreover, there are many instances in man where specific tumors do not contain mutations within the p53 coding region. Instead, mutations that block p53 transcription or mutations in genes that regulate p53 protein activity (e.g., mdm2) are responsible for the loss of p53 function. For example, overexpression of p53, indicative of p53 mutation or stabilization of the protein, is common in human histiocytomas. Without knowing the levels of p53 RNA and protein in the cell, it is not possible to know whether p53 is functional in our investigated tumors. Nonetheless, our data clearly suggest a rare occurrence of such mutations in the canine hemangiopericytoma, mastocytoma, and histiocytoma.

In human oncology, the causal importance of all or at least some chromosomal aberrations in the tumorigenic process.
has been established beyond doubt (Heim and Mitelman 1995). In parallel with the deepening of basic biologic understanding of neoplastic mechanisms, the clinical usefulness of various cytogenetic abnormalities as diagnostic and prognostic aids has been increasingly appreciated. Initially this applied only to hematologic malignancies, but now the diagnostic karyotype also plays a role in the evaluation of many solid tumors (Heim and Mitelman 1995).

Hemangiopericytomas, mastocytomas, and histiocytomas are relatively frequent tumors in dogs, at least in certain breeds (Yager and Scott 1993). In contrast, chloromas are very rare in dogs. Because of the total lack of chromosome banding data on mastocytomas, histiocytomas, and chloromas, the type of causal relationship of the observed chromosome abnormalities is hard to evaluate. Future studies on further patients will elucidate their contribution to tumor initiation and tumor promotion. The finding in the case of hemangiopericytoma provides further evidence for the nonrandom participation of trisomy 2 and centric fusions (Mayr et al. 1992, 1995) in the development of this tumor type.

Further studies on genetic abnormalities in canine neoplasms will lead to a better understanding of these diseases and provide valuable clinical tools for veterinary and comparative oncology.

References

Figure 7. Karyotype of dog 3. Granulocytic sarcoma. 2n = 78. Note the presence of trisomy 5 and monosomy 31 (arrows).

Figure 8. Karyotype of dog 4. Hemangiopericytoma. 2n = 78. The arrows indicate trisomy 2 and the centric fusion 19:21.


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