ANTICANCER RESEARCH *25*: 3995-3998 (2005)

Cytogenetic Investigations in Four Canine Lymphomas

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Abstract. *Four cases of canine lymphoma are presented, including histological examination and cytogenetic investigation. The first case showed a derivative chromosome 13, the second case showed a clonal trisomy 8 and the third case showed a complex karyotype with a clonal trisomy 13 and additional clonal trisomies of the chromosomes 20, 30 and 37, as well as a non-clonal tetrasomy 9. Case four showed a single trisomy 2. Comparing these results with human hematopoietic malignancies, there are notable similarities between both species.*

A number of genetic alterations for hematopoietic diseases in humans have been described. Often, these alterations are specific chromosomal abnormalities, making cytogenetic analyses on outstanding tool for the diagnosis of these diseases. To date, only a few reports exist about cytogenetic investigations of hematopoietic diseases in dogs. This is certainly due to the difficult karyotype of the dog, which is comprised of 76 small acrocentric autosomes. However, there are some papers describing cytogenetic changes in canine cancers (1-13). On the one hand, these investigations provide a comparison with corresponding findings in humans and, on the other hand, they can help to improve diagnosis in veterinary medicine and facilitate prognosis about progress of the disease. Diseases of the hematopoietic and the lymphatic system, in particular, are among the most frequently observed malignant neoplasms in dogs (14). Thus, canine lymphosarcomas, the clinical appearance, histopathology and treatment of which are comparable to human non-Hodgkin's lymphomas, are found in approximately 0.36% of all dogs receiving veterinary care. Thus, the canine lymphosarcoma accounts for about 83% of all hematological malignancies of the dog (15). In a study by Hahn *et al.* (15) on canine lymphosarcomas, trisomies of chromosomes 13, 34 and 36 were found. In previous investigations, we were able to show two cases of leukemia in which trisomy 1 was present. One case consisted of a simple trisomy of chromosome 1, and the other case of centric fusion of two additional chromosomes 1 (8-10). Herein, the karyotypic alterations detected in four dogs with different types of lymphomas are described.

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Key Words: Canis familiaris, cytogenetics, lymphoma.

Case Report

Four dogs, admitted to the Clinic for Small Animals, School of Veterinary Medicine, Hannover, Germany, were clinically, cytologically and cytogenetically examined in detail. They included a 5-year-old male Munsterlander (Case KM 15) and a 4-year-old female Bernese Mountain Dog (case KM 115) both with centroblastic lymphomas stage IV, a 4-year-old male German Shepherd dog (case KM 39) with a centroblastic lymphoma stage V and a 6-year-old male Golden Retriever (case KM 29) with an immunoblastic B-cell lymphoma stage V.

Materials and Methods

Bone marrow samples were taken from the iliac crest of the dogs and immediately transferred to 1 ml sodium heparin. The marrow cells were centrifuged at 135 x g and incubated for 48 hours in McCoy's medium. Subsequently, colcemide (0.1 μg/ml) was added for 2 hours. The cells were centrifuged again at 135 x g for 10 minutes and incubated for 15 minutes in 0.05 M KCl. Finally, the cells were fixed overnight with methanol/glacial acetic acid. This suspension was dropped on ice-cold slides and dried for at least 7 days at 37°C. The chromosomes were stained by GTG banding, and the karyotype was described following the nomenclature of Reimann *et al.* (16). The description of the cytogenetic aberrations was carried out according to the instructions of the "International System for Human Cytogenetic Nomenclature (1995)" (17).

Results

As listed in Table I, cytogenetic investigation of the bone marrow showed a derivative chromosome 13 in case KM 15,

Table I. *Karyotype description of the four cases of canine lymphomas examined. Brackets show the number of metaphases with similar findings, bold type summarizes clonal changes.*

Case	Histological diagnosis	Karyotype
KM 15	Centroblastic lymphoma stage IV	78, XY [1] **78, XY, der (13) [2]** 77, XY, –36 [1] 78, XY, –13, +mar [1] 78 [3] 75 [1] 77 [2]
KM 29	Immunoblastic B-cell lymphoma stage V	79, XY, +8 [3] 79, XY, der(4), der(7), +8 [2] 79, XY [3]
KM 39	Centroblastic lymphoma stage V	**84, XY, +9, +9, +13, +20, +30, +37 [3]** **84, XY, +9, +9, +13, +16, +24, +31 [1]** **84, XY, +9, +13, +20, +30, +36, +37 [1]** **82, XY, +9, +13, +20, +37 [1]** 84 [1] 86 [1] 87 [1]
KM 115	Centroblastic lymphoma stage IV	78, XX [1] **78, XX, +2, –29 [1]** **79,XX, +2 [1]** 77 [2] 78 [3] 79 [4] 80 [1]

that was present in 2 out of 5 metaphases for which karyotype descriptions were performed, representing the primary aberration. In this case, the derivative chromosome 13 obviously shows an increase of chromosomal material. In case KM 39, a clonal trisomy 13 was observed as well, in conjunction with a complex karyotype showing additional clonal trisomies of chromosomes 20, 30 and 37 and a clonal tetrasomy 9. Case KM 115 showed a clonal trisomy 2. Case KM 29 showed a clonal trisomy 8, that was found in all metaphases investigated. Additionally, two derivative chromosomes, 4 and 7, were present in two metaphases, indicating another clonal aberration.

Discussion

Although to date little is known about chromosomal changes in hematopoietic diseases and solid tumors of the dog, some differences in comparison to those found in humans are remarkable, such as the overall low frequency of specific translocations in canine neoplasias. Aberrations of chromosome 13 were found in two cases of the present study (KM 15, KM 39), including one case with trisomy 13 (Figure 1, Figure 2B). Concerning canine lymphosarcomas, trisomy 13 has been described earlier. In a study of 61 dogs with lymphosarcoma, trisomy 13 was found in 15 cases (15). Cytogenetic changes with involvement of chromosome 13 were even considered the most frequent clonal changes in canine lymphosarcomas. Dogs that exhibited trisomy 13 as the primary aberration in a lymphosarcoma showed a significantly longer duration of the first remission and of survival compared to animals with other chromosomal changes, because they responded better to a given chemotherapy (15). Thus, changes of the canine chromosome 13 may turn out to be a suitable marker for diagnosis and prognosis of canine lymphosarcomas. With regard to homology to human chromosomes, Yang *et al.* (18) showed homologies between CFA 13 and a comparatively long segment of HSA 8 and two small segments on HSA 4. The appearance of trisomy 8 in human lymphomas and myeloid leukemias, as well as the appearance of trisomy 4 in acute myeloid leukemias, supports the assumption that, in both species, the generation of cancer is comparable, even though there is a lack of structural chromosomal aberrations in canine hematopoietic neoplasias. Up to now, chromosomal fusions as well as translocations have not been found frequently in dogs. However, in addition to aberrations of chromosome 13, other aberrations detected in the present study are similar to those described earlier in solid or hematopoietic tumors of the dog. The clonal trisomy 2 of case KM 115 shows similarities to cases already described in the literature. In solid canine tumors, aberrations of chromosome 2 have been reported repeatedly (19, 20). Again, compared to human chromosomes, Yang *et al.* (18) showed homologies of CFA 2 to a relatively long segment on HSA 10 and a smaller segment on HSA 1. In human leukemia and lymphoma, trisomy 10 is sometimes observed in myeloid leukemia.

The fourth case in the present study resembles the cases described earlier in this paper. In case KM 29, a clonal trisomy 8 was found (Figure 2A). According to Yang *et al.* (18), CFA 8 was nearly identical to human chromosome 14. Again, trisomy 14 often occurs in human myeloid leukemias and malignant lymphomas, with a main focus in myeloid disorders. Some cases are reported in which trisomy 14 is due to an isochromosome 14 (21). The human oncogene *BCL-1* is located on the long arm of chromosome 14 at band q32. Translocations affecting this chromosomal region represent a common mechanism of oncogene activation in human malignant lymphoid malignancies, whereas in human multiple myeloma, the most frequent chromosomal aberration is a 14q+ marker (22).

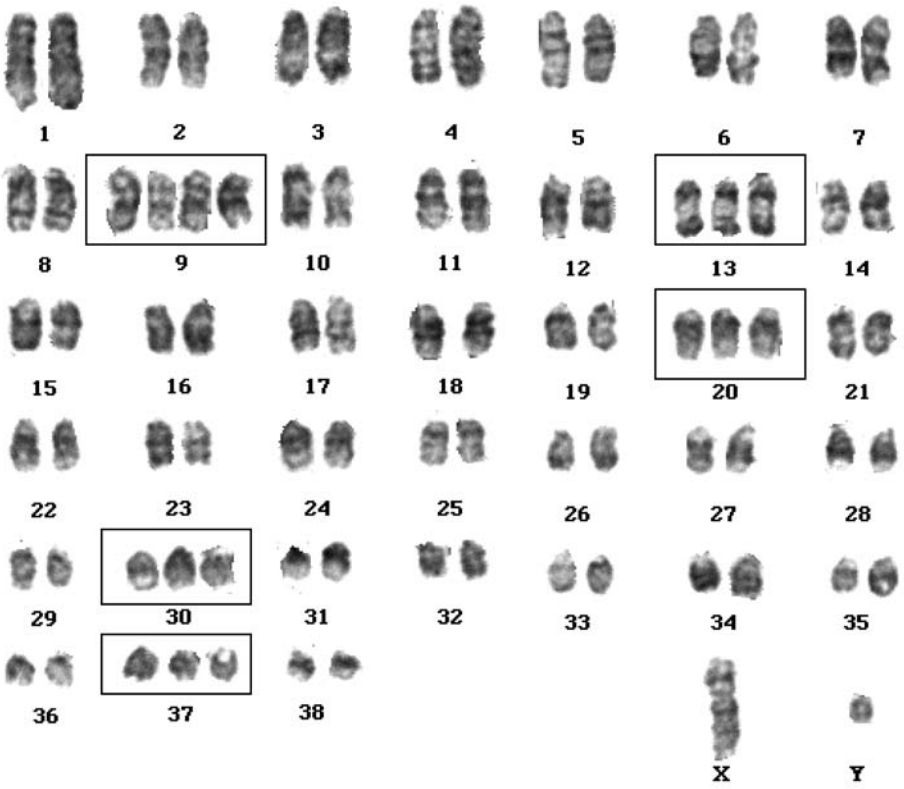

Figure 1. *The complex karyotype of KM 39: 84, XY, +9, +9, +13, +20, +30, +37.*

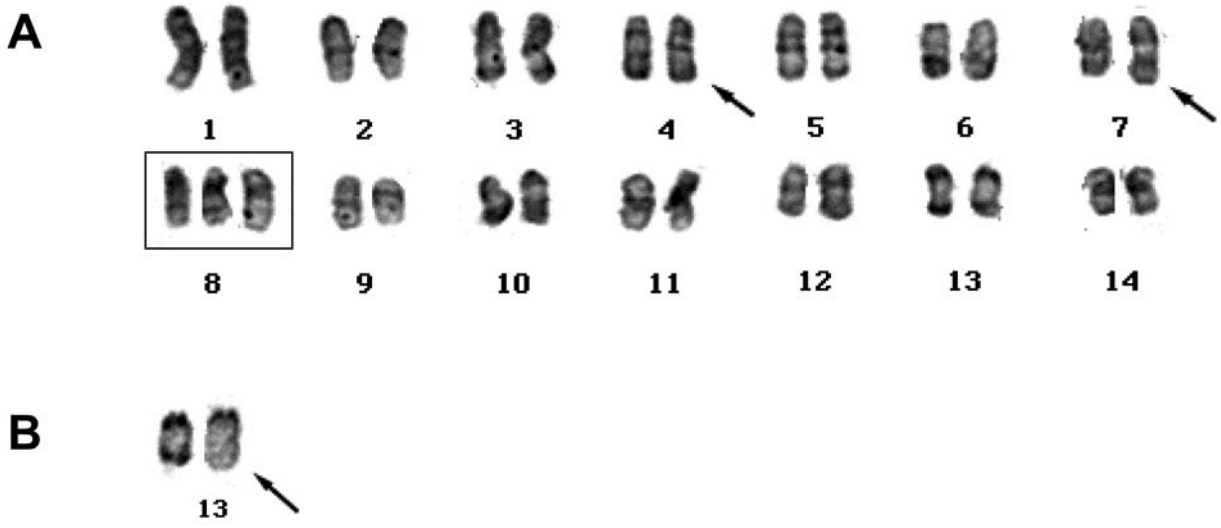

Figure 2. *Partial karyotypes. A: representative chromosomes 1 – 14 of KM 29. B: representative chromosome 13 of KM 15.*

Comparing the results of the present study with findings in human hematopoietic diseases, there are striking similarities in both species regarding cytogenetic alterations involved in the development of cancer. Thus, the dog may serve as an animal model for cancer research and drug discovery, thereby also taking advantage of improved therapy.

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Received April 25, 2005
Accepted June 30, 2005