**Short Communication**

**Canine lymphomas: DNA changes in tumour genes**

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Lymphoma is the most frequent hematopoietic neoplasm in dog, and it shares some clinical, cytological, histopathological and molecular similarities with humans. In our study, DNA changes in *TP53*, *c-KIT* and *N-RAS* were detected in lymphoma tissues of cross breed bitch and Bernese Mountain Dog bitch. One transition A/G at nucleotide position 138 in exon 5 in *TP53* gene and one transition G/A at nucleotide position 35 in exon 8 of *c-KIT* gene were observed in donor 2. It seems that these genes did not play a key role in canine lymphoma formation and progression.

**Keywords:** canine lymphoma, *TP53*, *C-KIT*, *N-RAS*

# Introduction

Lymphoma represents a heterogeneous group of neoplastic blood disorders involving monoclonal proliferation of malignant lymphocytes. Historically, lymphomas have been divided in two basic categories: Hodgkin lymphoma (HL) and Non-Hodgkin lymphoma (NHL) (DeVita et al., 2015). Different subtypes were described in current WHO classification (WHO, 2008), which is based on various biological and clinical features of the disease. In humans, 5.1 % of all cancer cases was diagnosed as NHL and 2.7 % of all cancers had been a cause of death. (Boffetta, 2011).

Numerous chromosomal imbalances have been documented in human lymphomas (Hallermann et al., 2004). For example, translocation t(14;18) have been recorded by D´Haese et al. (2005) after sequencing of the *BCL2* gene in patients with follicular non-Hodgkin´s lymphoma. Mutations in a lymphoid tissue have also been observed in mice. DNA changes in five genes (*Bcl11b, Ikaros, Myc, Pten and Notch1*) have been documented by Ohi et al. (2007) in mice with thymic lymphomas after γ-irradiation In this paper the potential DNA changes in two protooncogenes (*N-RAS* and *c-KIT*), and *TP53* tumour suppressor gene were analyzed in canine lymphomas.

# Material and methods

## Isolation of test and reference DNA

The tested genomic DNA was isolated from the tumour tissues (lymphomas) from two 10- and 12-years old bitches. One dog (donor 1) was a cross breed bitch (with a solid tumour in a node) and the second (donor 2) was Bernese Mountain Dog bitch (in a final stage of cancer with numerous metastases). The DNA reference was isolated from the whole blood of healthy dog (Jack Russell Terrier dog, 7 years old), using the DNeasy® Blood & Tissue Kit (Qiagen, Venlo, Netherlands).

## Design of primers and PCR reaction

Specific primers were designed for *Canis lupus familiaris* genes as follows: for *TP53* gene,exons 5, 6(ENSCAFE00000181392, 187bp andENSCAFE00000181396, 113bp; including intron), and exons 7, 8 (ENSCAFE00000181397, 110bp and ENSCAFE00000181399, 137bp; including intron), for *c-KIT,* exon 8 (ENSCAFE00000022604, 115bp) and exon 17 (ENSCAFE00000022614, 123bp) and for *N-RAS,* exon 1 (ENSCAFE00000103409, 128bp) and exon 2 (ENSCAFE00000103411, 179bp) by Primer3Plus software. PCR reaction mixture consisted of 10 µl PCR Master Mix (Promega M 750, Fitchburg, WI, USA); 0,4 µl primers; 0,5 µl tested DNA and 9,1 µl PCR Ultra H2O (Top Bio, Prague, Czech Republic) with the total volume of 20 µl. The standard PCR programme included five steps. Initial DNA denaturation phase starting at 95oC for 5 min was followed by subsequent repetition of 30-35 cycles: 30 s at 95oC for DNA denaturation, 45s at variable temperatures (54-61oC) for primer annealing, 45 s at 72oC for amplification. Final extension step was set for 45 s at 72oC and followed by rapid cooling to 4oC. The products of PCR reaction were analysed by agarose gel electrophoresis (1,5%) with GelRed (Biotinum, Hayward, CA, USA) staining and visualization under UV illumination - GenoView Smart M (VWR GenoView, Radnor, PA, USA).

## DNA sequencing

The PCR amplicons of all gene exons were sequenced by ABI PRISM 3100-Avant Genetic Analyzer (Applied Bio-systems, Waltham, MA, USA) (Laboratory of Biomedical Microbiology and Immunology, UVLF in Košice). The results of sequencing were compared with the reference sequences in GenBank and for *TP53* evaluated by the DNASTAR program under the accession number NM\_001003210, and for *c-KIT and N-RAS* in NCBI under the accession numbers AY313776.1 and NM\_001287065.1.

# Results and discussion

**Cancer is the result of several genetic events in somatic cells, and in some cases the result of predisposition to inhered mutations in the responsible genes.** Studied genes *TP53*, *c-KIT* and *N-RAS* were chosen based on our previous results of the CGH analysis, by which a number of imbalances on the canine chromosomes (both cross breed bitch and Bernese Mountain Dog) were detected(Drážovská et al., 2016).

Nucleotide sequences of ten amplicons (shown in Fig. 1) were compared with reference sequences. A total, only two nucleotide substitutions (transitions) were identified in donor 2; One A/G at nucleotide position 138 in exon 5 in *TP53* gene (Fig. 2), and the second one, G/A at nucleotide position 35 in exon 8 of *c-KIT* gene (Fig.3).



Figure 1 Electrophoresis of PCR amplicons of genes *TP53*, *C-KIT* and *N-RAS* (DNA ladder-100bp); D1 - donor 1; D2 - donor 2

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Figure 2 Sequencing of exon 5 TP53. Transition A/G at position 138 in Bernese Mountain Dog bitch (donor 2)



Figure 3 Sequencing of exon 8 c-KIT. Transition G/A at position 35 in Bernese Mountain Dog bitch (donor 2)

The *TP53* is a key of the tumour suppressor genes controlling cell cycle proliferation, mutations in which are most frequently described in association with numerous cancer diseases. Single base-pair missense mutations in *TP53* gene have been most frequently identified in humans. Brathwaite et al. (Brathwaite et al., 1992) described several mutations in exons 5, 7 and 8 in murine thymic lymphomas; G:C to A:T transitions were also occurred more frequently. In contrast to these findings, no point mutations in exons 5-8 of the *p53* gene were recorded by Hollstein et al. (1997) in 13 iatrogenic human liver cancers or in canine brain tumours (York et al., 2011), as well.

The proto-oncogene *c-KIT* plays an important role in proliferation, survival and differentiation of hematopoietic progenitor cells. In humans, c-kit expression has been well documented in different hematopoietic neoplasms, like acute myeloid leukemia, granulocytic sarcoma, systemic mastocytosis, T-cell acute lymphoblastic leukemia and multiple myeloma; contradictory results have been reported in lymphomas.

The *RAS* genes (N, K and H) encode proteins important in cell signal transduction. Although missense mutations in *N-RAS* gene were detected by Usher et al. (2009) in 25% dogs with acute lymphoid leukaemia, our results indicated that ***N-RAS* gene activation in canine lymphoma is rarely, like as considered Mayr et al. (2002).**

# Conclusions

Canine lymphoma (cL) represents the most common hematopoietic neoplasia in dog and involves many similarities in clinical expression, molecular mechanisms, treatment and drug response with human NHL (15). Only two nucleotide substitutions (transitions) were found in *TP53* and *c-KIT* genes in donor 2. Position of genes on canine chromosomes is not known. We assume that studied genes were either not located in the place of detected chromosome imbalances (Drážovská et al., 2016) or chromosomal rearrangements did not lead to the changes in their activity. Probably these genes did not play a key role in canine lymphoma formation and progression.

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