Artykuł przeglądowy *Review*

Nuclear and mitochondrial DNA mutation in human and canine tumors

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Summary

Over the past few years, there has been a significant progress in genetic testing in dogs through the development of genomic maps, which make it possible to determine the background of polygenic genetic diseases. The information obtained by mapping the canine genome, a detailed marker map, and the understanding of genome architecture have changed the possibilities and direction of canine genetic research. It is now possible to understand the genomics of the qualitative and quantitative traits associated with the phenotype, genetic predisposition, and genetic background of canine defects and diseases.

Investigations of canine genetic diseases are particularly valuable because their results can be used in human medicine. From the medical point of view, genetic diseases in both humans and dogs are similar, which makes it possible to test new therapeutic approaches in the case of orthologous genes containing mutations responsible for genetic disorders.

Keywords: mutations, genes, nDNA, mtDNA, cancer

Over the past few years, there has been a significant progress in genetic testing in dogs through the development of genomic maps, which make it possible to determine the background of polygenic genetic diseases. The information obtained by mapping the canine genome, a detailed marker map, and the understanding of genome architecture have changed the possibilities and direction of canine genetic research. It is now possible to understand the genomics of the qualitative and quantitative traits associated with the phenotype, genetic predisposition, and genetic background of canine defects and diseases.

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Cancer: definition and classification

There is no uniform definition of cancer that would exhaustively describe all phenomena associated with the disease. There are many definitions of tumor, which to a greater or lesser extent reflect the nature of the pathological process. For the last 50 years the most commonly used and accepted cancer definition has been the one proposed by Willis (50). Cancer is a pathological tissue growing in an excessive and uncoordinated way with excessive proliferation that is sustained even after the causative factor has been eliminated.

The basis for the classification of tumors is their tissue of origin. According to histogenetic criteria, one group of cancers are tumors of epithelial origin, derived from each type of epithelial cells, such as glandular and covering cells, cells lining the respiratory, urinary, and digestive tracts, the reproductive system, as well as neuroectodermal cells. The other group are tumors of mesenchymal origin, which include all

tumors of connective, hematopoietic, vascular, and muscular tissues. Histogenetic classification is also included in the histological nomenclature of tumors. Carcinoma is a malignant neoplasm derived from epithelial cells, and sarcoma is a malignant tumor of mesenchymal origin (5).

Hereditary predisposition to neoplasia

A number of genes whose mutations are responsible for hereditary predisposition to human and canine cancer have already been identified. Most of them are located in nuclear DNA (nDNA), but some have also

Tab. 1. Selected nuclear genes whose mutations predispose to human and canine cancer

Human			Dog	
Gene	Location/ chromosome	Predisposition to cancer	Location/ chromosome	Predisposition to cancer/Gene ID
BRCA1 (breast cancer 1) (11, 27, 37, 49) MIM number 113705	17q21.31	breast cancer, ovarian cancer, malignant lymphoma, prostate cancer, colorectal cancer, fallopian tube cancer, pancreatic cancers, malignant melanomas	9	breast cancer (36)/403437
BRCA2 (breast cancer 2) (37, 49, 51) MIM number 600185	13p11.2		25	breast cancer (18, 36)/474180
VHL (von Hippel-Lindau tumor suppressor) (30) MIM number 608537	3p25.3	hemangiomas of cerebellum and retina, renal cell tumors, adrenal gland tumors	20	renal cell carcinoma (34)/494000
MSH2 (mutS homolog 2) (8, 26, 37) MIM number 609309	2p21	colorectal cancer, uterus cancer, gastric cancer, small intestine cancer, renal cell tumors, bladder cancer, bile duct cancer, ovarian cancer	10	canine cutaneous mast cell tumors (28)/494002
MLH1 (mutL homolog 1) (26) MIM number 120436	3p22.2		23	no data/477019
MSH6 (mutS homolog 6 (E. coli)) (26) MIM number 600678	2p16.3		10	no data/474585
FGFR2 (fibroblast growth factor receptor 2) (9, 19) MIM number 176943	10q26.13	breast cancer	28	breast cancer? (36)/415125
TOX3 (TOX high mobility group box family member 3; TNRC9, trinucleotide repeat--containing 9) (9) MIM number 611416	16q12.1	breast cancer	2	no association with breast cancer (36)/487281
CHEK2 (checkpoint kinase 2) (49) MIM number 604373	22p11.1	breast cancer	26	no association with breast cancer (36)/486338
MAP3K1 (mitogen-activated protein kinase kinase kinase 1) (9) MIM number 600982	5q11.2	breast cancer	2	no association with breast cancer (36)/478061
LSP1 (lymphocyte-specific protein 1) (9) MIM number 153432	11p15.5	breast cancer	18	no association with breast cancer (36)/611553
RCAS1 (estrogen receptor binding site associated) (40, 42) MIM number 605772	8q23.2	various cancers, including uterus, ovarian, lung and breast cancer	13	no association with breast cancer (36)/403500
TP53 (tumor protein p53) (37, 49) MIM number 191170	17p13.1	breast cancer, soft tissue sarcomas, leukemia, brain tumors	5	no association with breast cancer (36)/403869
ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, gene encoding HER2) (33) MIM number 164870	17q21.2	breast cancer	9	no association with breast cancer (36)/403883
RAD51 (homolog (S. cerevisiae) (15, 38) MIM number 179617	15q11.2	head and neck cancer, breast cancer	30	simple adenomas and adenocarcinomas mammary gland (22, 23)/403568
Rb/Rb1 (retinoblastoma/1) MIM number 180200	13q14	retinoblastoma	22	retinoblastoma (47)/476915
PTEN (phosphatase and tensin homolog) MIM number 158350	10q23.31	Cowden syndrome	26	Colorectal hamartomatous polyposis and ganglioneuromatosis (1)/ 403832/MIA number 001515-9615
KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) MIM number 606764	4q12	Gastrointestinal stromal tumor	13	Gastrointestinal stromal tumor (14)/ 3815/ MIA number 001516-9615
FLCN (folliculin) MIM number: 135150	17p11.2	Birt-Hogg-Dube syndrome (including renal cell tumors)	5	Renal cystadenocarcinoma and nodular dermatofibrosis (25)/ 479529/MIA 001335-9615

been identified in mitochondrial DNA (mtDNA). It is estimated that the risk of cancer disease in carriers of nuclear gene mutations may be as high as 80-90%. It should be emphasized that this defines the likelihood of the occurrence of cancer disease in the life of a carrier of the mutation, i.e. the so-called gene penetration.

Currently, in human medical practice, the most frequently tested nuclear genes related to hereditary cancer include (Tab. 1) Rb, with penetration at the level of ca. 90%, BRCA1 and BRCA2 (ca. 80%), VHL (about 80%), MSH2, MLH1, and MSH6 with gender--dependent penetration: ca. 90% in men and 70% in women (8, 26, 27, 30, 51).

The examination of the genetic background of cancers in humans is obviously a matter of priority worldwide because of the increase in their incidence and mortality. The results of studies of human tumors are far more advanced in comparison with the results of similar research on dogs. This is why veterinary research aimed at the assessment of dogs' susceptibility to tumor diseases draws on the achievements of human medicine.

In the available literature there is little information on the occurrence, frequency and importance of mutations, and on their role in the development and malignant transformation of canine tumors (Tab. 1). This is due to the fact that the canine genome has been sequenced and mapped only recently. Currently, there are plans to collect a greater number of canine cancer samples in order to obtain reliable results concerning the relationship between individual mutations and canine cancer, as well as the relationship between dog and human genetics and cancer treatment in humans. This is one of the main tasks of the LUPA Consortium initiative.

The Consortium is planning to identify the main genes of several diseases, including four cancers, within a few next years. The research is designed to examine eight thousand dogs by conducting GWAS. The identification of polygenic traits will be an enormous challenge. The accuracy of the estimates of its genetic determination will concern monogenic and autosomal dominant traits (http://www.eurolupa.org/).

The dog as a species model of human cancers

The latest research is focused primarily on the identification of causative mutations leading to the formation of human and canine cancers. In the future, these tests may become the basis for the development of new therapeutic approaches in both species.

The dog has become a promising model for the study of human genetic diseases, including cancer, which leads to increased interest in canine genomics (43, 44).

Four catalogues of recognized inherited canine diseases are available for free on the Internet (43). They provide important information regarding the prevalence and genetic determinants of theses diseases. Almost 370 canine genetic disorders similar to the ones occurring in humans have been described (IDID, http://www.vet.cam.ac.uk/idid). Only few of them, however, have been identified at the molecular level. To date, 583 genetic defects, including 153 diagnosed at the molecular level, have been described in dogs. Until no. 298, they are genetic diseases, in which the dog may become a potential model for human genetic diseases (OMIM, http://omim.org), of which tumors are only a small proportion (http://omia.angis.org.au/; accessed 04.11.2012).

In 2005, Lindblad-Toh et al. (24) identified the canine genome sequence. It was the beginning of the comparative study of the molecular background of canine and human genetic diseases. This was related the fact the human and canine genome sequences were found to be highly homologous. Comparative genomic investigations demonstrating interspecific gene homologies indicate that 93% of genes in the human and canine genomes are orthologous genes (13). Owing to the fact that the mapping of the human genome was provisionally completed a few years earlier, knowledge about malignant transformation in dogs is scantier than the corresponding information concerning man. Nevertheless, the dog as a model for studying the background of human cancer has important advantages over the artificially induced mouse model. Dog breeders endeavor to reduce the prevalence of tumors in different breeds of dogs. Furthermore, knowledge about the welfare of companion animals is increasing along with the ethical concerns about the use of laboratory animals. The constantly growing number of studies and publications on the molecular background of canine tumors provides evidence for the development of veterinary oncology (37, 43). This gives grounds to believe that the dog can be a model in studies of tumors in humans, as well as in various species of livestock (43, 44).

Human and canine mammary tumors

Mammary tumors are the most commonly occurring cancers in female dogs (*Canis familiaris*). Mammary tumors account for about one half of all cancers in females, and about one half of the cases of canine mammary tumors are malignant. In women and dogs, the probability of the occurrence of mammary tumors increases with age: they are rarely diagnosed before 25 and 5 years of age, respectively. In dogs, canine mammary tumors occur most often between 10 and 11 years of age, but in some breeds, they occur at a younger age. For instance, the average age of occurrence in the English Springer Spaniel is 7 years (4, 10, 37). In terms of epidemiology, clinical manifestation, morphological traits, and prognosis, canine mammary carcinoma is similar to human breast cancer. Therefore, in this respect, the dog is an appropriate animal model with naturally occurring tumors (36).

Breast cancer is the leading cause of morbidity and mortality in women. Several genes predisposing to its

development have been identified (Tab. 1), but most of the malignant transformation risk factors still remain unknown. Even less is known about hereditary risk factors in canine mammary tumors. Some breeds are particularly exposed (predisposed) to its occurrence, for example the English Springer Spaniel in Sweden, where 36% of these dogs are affected (10).

Rivera et al. (37) conducted their research on dogs affected by mammary cancer and analyzed sequences of 10 genes whose mutations had been described in female breast cancer (BRCA1, BRCA2, CHEK2, ERBB2, FGFR2, LSP1, MAP3K1, RCAS1, TOX3, and TP53). They described 63 single-nucleotide polymorphisms (SNPs, 4-9 per gene). They found that the genes BRCA1 and BRCA2 were associated with a significantly increased mammary tumor risk in a breed with known prevalence of mammary tumors, that is, in the English Springer Spaniel. A borderline association was observed for FGFR2, but it was lost after the Bonferroni correction. By analyzing benign and malignant changes separately, they found a significant relationship in the case of both BRCA1 and BRCA2, with a stronger interdependence between BRCA1 and malignant tumors. The other selected genes showed no association with an increased risk of canine mammary tumors in the test population. The investigations indicate that the BRCA1 and BRCA2 genes contribute to the risk of canine mammary tumors, which suggests that dogs can be a model of female breast cancers (37).

Different levels of the expression of BRCA1, BRCA2, and RAD51 in simple adenomas and adenocarcinomas of the canine mammary gland were reported in studies by Klopfleisch and Gruber (22). They found that the gene complex of tumor suppressor genes plays a role in the DNA damage response. In adenomas, gene expression was reduced in the case of all genes investigated. In adenocarcinomas, an increase in the expression of BRCA1 was reported in a few cases, whereas overexpression was detected in the case of BRCA2 and RAD51. The results of these authors suggest that RAD1 is involved in most canine mammary carcinomas and lymph node metastases of canine mammary tumors (22, 23).

Hsu et al. (18) studied BRCA2 exon 11 in canine mammary tumors. They found a relationship between the clinicopathologic status and point mutations (SNP) in BRCA2 (2414 G > A and 511 and > C). Both „hot spots" were detected in most types of cancer and tumor stages. The authors suggest that hot spots may be used as prognostic factors for malignant neoplasms and malignant mammary tumors in dogs. However, the results should be treated with caution because of the small number of samples included in the study.

Other human and canine cancers

In addition to the increased risk of breast cancer, most tumor suppressor genes are associated with cell proliferation and differentiation processes, as well as with other human cancers. These include ovarian cancer (ovarian carcinoma cell), malignant lymphoma, prostate cancer, fallopian tube cancer, pancreatic cancers and malignant melanomas (BRCA1/2), soft tissue sarcomas, leukemia and brain tumors (TP53), hemangiomas of cerebellum and retina, renal cell tumors, adrenal gland tumors (VHL), colorectal cancer, uterus cancer, gastric cancer, small intestine cancer, renal cell tumors, bladder cancer, bile duct cancer, ovarian cancer (MSH2, MLH1, MSH6), uterus, ovarian and lung cancers (RCAS1), head and neck cancer (RAD51) retinoblastoma (Rb), Cowden syndrome (PTEN), gastrointestinal stromal tumor (KIT), and Birt-Hogg-Dube syndrome (FLCN).

As mentioned before, studies on genetic determinants of canine cancer are less advanced than those on human cancer, so much fewer mutations associated with carcinogenesis in dogs have been described. Although sequences of several genes have been studied, their impact on malignant transformation has been specified only in a few cases. This applies to breast cancer (BRCA1/2 and probably FGFR2), renal cell carcinoma (VHL), canine cutaneous mast cell tumors (MSH2), simple adenomas and mammary gland adenocarcinomas (RAD51), retinoblastoma (retinoblastoma protein), colorectal hamartomatous polyposis and ganglioneuromatosis (PTEN), gastrointestinal stromal tumor (KIT), and renal cell tumors (FLCN) (Tab. 1).

It should be mentioned that in several cases mutations in the same human and canine genes were associated with the same tissues (BRCA1, BRCA2, VHL, RAD51, Rb, PTEN, KIT, FLCN). This may confirm the possibility of using the dog as a model for investigations of human breast cancer, renal cell carcinoma, colorectal polyposis hamartomatous retinoblastoma, and gastrointestinal stromal tumor (Tab. 1).

Multimarker gene expression studies

The first multimarker gene expression studies of canine mammary tumor and malignant lymphoma were carried out with the use of two human cDNA microarrays in 2005 (11). This was due to the unavailability of the canine microarray, but the results also confirmed a high homology of encoding gene sequences. Currently, whole canine genome expression arrays are available. They were first used in a study conducted by Rao et al. (35). The cDNA arrays contained almost 21 thousand canine genes. In the study, the following cell lines were used: CMT-U335 (canine mammary osteosarcoma), CMT-U229 (canine mammary atypical benign mixed tumor), and P114 (highly malignant canine anaplastic carcinoma). The results of the study pointed to the existence of both overexpression and reduced gene expression involved in the molecular pathways of the cell, partly converging for lines CMT-U229 and P114, but divergent in comparison with the aggressive osteosarcoma CMT-U335 (35).

Uva et al. (46) analyzed gene expression in healthy tissue and in canine and human mammary tumor. The experiments involved a genome-wide comparative analysis of transcriptional changes. On the basis of the analysis of the expression of about one thousand orthologous canine and human genes, they observed significant similarity in the deregulation of genes in samples from breast tumors, compared with their counterparts in healthy tissues. The analysis of gene expression revealed a high degree of similarity in canine and human disorders in the case of many tumor-dependent pathways (PI3K/AKT, KRAS, PTEN, WNT-beta catenin, and MAPK cascade). In addition, transcriptional relationships between various genes observed in human breast cancer were largely identical in the dog, which suggests a close interspecific similarity of the background of canine and human mammary cancer. This constitutes additional evidence for the importance of the canine model in studies of human tumors and for the possibility of seeking new diagnostic and prognostic biomarkers that can be used in clinical trials (46). Canine mammary tumors are an attractive alternative to the classical model of transgenic or xenogenic mice, in which cancer is induced artificially.

Genome-wide association study (GWAS)

An intensive development of technology and bioinformatics has facilitated the development of microarrays for a rapid determination of the genotype at tens of thousands of loci. Hence, genome-wide association studies (GWAS) have become possible.

Using a genome-wide association study, Easton et al. (9) found four genes (FGFR2, TNRC9, MAP3K1, and LSP1) that were significantly associated with an increased risk of human breast cancer. RCAS1 also had a significant effect on the development of various cancers, including lung, ovary, and uterus tumors (40).

BRCA1 and BRCA2 gene mutations do not account for most of the hereditary cases of breast tumors. A great deal of research has been done on the basis of linkage analysis in order to identify other genes/regions of chromosomes. In some of the first GWAS, the 4.720 SNP array was used to scan the genome. Investigations have indicated 6 regions on chromosomes containing candidate genes involved in breast cancer. Ongoing analyses performed with multi-NAP arrays still indicate regions of the genome containing 300 genes related to the risk of breast cancer (2, 39). Some of the identified genes associated with an increased risk of breast cancer include checkpoint kinase 2 (CHEK2), fibroblast growth factor 2 (FGR2), transformation-related protein 53 (TP53), and phosphatase and tensin homology (PTEN). They are involved in the process of mutagenesis as corrective or suppressor genes. Currently, genes involved in cell proliferation pathways, that is, FGFR2, mitogen-activated protein kinase, RAD50 1 (MAP3K1), and the transforming growth factor b1 (TGFB1) are assumed to be related to malignant transformation (9, 19, 42, 49).

Currently, microarrays are available for the analysis of the polymorphism SNP of the genome of various livestock species, including the dog (more than 170 000 SNPS) (www.illumina.com), which facilitates genome-wide association studies (GWAS) in this species. Owing to the presence of many genetic diseases in dogs, the species is a perfect object for disease gene mapping. Two hereditary disease phenotypes of Rhodesian Ridgebacks have recently been mapped by using a genotyping array with about 27,000 SNPs (20).

MtDNA mutations in cancer

Mitochondrial DNA (mtDNA) sequence analysis is an important element used in population genetics and medicine. The development of automatic sequencing technology and bioinformatic methods has contributed to the development of mitochondrial genomics (41, 43).

The role of mitochondria in the malignant process was determined already over 70 years ago, when, in 1956, Otto Warburg described the glycolysis phenomenon occurring in cancer despite the presence of oxygen. The intensification of glycolysis occurs when the oxygen partial pressure decreases in the rapidly growing tumor, and hypoxia takes place. In subsequent stages, when the concentration of oxygen is again in the normal range, cancer cells may still exhibit an increased activity of glycolysis. A high level of glycolysis in the presence of oxygen (called the Warburg effect) results from genetic or epigenetic changes and is characteristic of most cancers. Metabolic changes, such as elevated levels of glycolysis and increased glucose consumption, play a significant role in the process of malignant transformation, increase the aggressiveness and invasiveness of the tumor, and act as antiapoptotic factors. This theory is supported by the widespread presence of mutations in the mtDNA of cancer cells, which suggests that they may be oncogenic agents. However, the exact role and mechanism of mtDNA mutation in the process of cancerogenesis are still not fully explained (6, 7, 16, 17, 32).

The knowledge of disorders occurring in the genetic material of cancer cells is mainly related to nuclear DNA (Tab. 1). However, there is a growing number of reports in which mtDNA damage is shown to be relevant in the neoplastic process. Hereditary mutations in the genes of polypeptides encoded in mitochondrial DNA can cause defects in the mitochondrial oxidative phosphorylation (OXPHOS) system. An increased production of reactive oxygen species (ROS), accompanying the improper function of the respiratory chain and caused by mutations, seems to have a particularly significant effect. The high frequency of mtDNA mutations results from the absence of the protective action of histones, a less efficient DNA repair system, and the proximity of the respiratory chain, which is the main source of ROS in the cell. The appearance of

mutations, or a deficiency or blockage of one of the respiratory chain links, leads to the malfunction of the respiratory chain, which results in an excessive production of ROS, and consequently in a severe oxidative stress in cancer cells. The consequence is a reduction in the efficiency of cells, tissues, and organs (16, 32).

The prevalence and severity of mitochondrial disorder symptoms depend on the ratio between normal and mutant DNA. The dominance of mutated mtDNA in the cell leads to energy production disorders in the oxidative phosphorylation process, which is later manifested in the form of clinical signs. These symptoms are progressive and depend on the type of mtDNA damage, the degree of heteroplasmy, and the sensitivity of the specific tissue disorder associated with cell respiration (6, 16, 32, 45).

A normal cell can contain both mutated and normal wild-type mtDNA, a condition known as heteroplasmy. Most changes within the mtDNA of a neoplastic cell are of homoplasmic nature, that is, all of the DNA molecules in the cell are the same. Changes in mtDNA may be either mutations or polymorphisms. Changes that occur in both blood and tumor cells in the same individual are considered as polymorphisms. Mutations are changes characteristic of cancer cells only and not re-occurring in the patient's blood (16, 17).

The mitochondrial genome of the dog (*Canis familiaris*) shows very large similarity to the human genome (16). Canine MtDNA has a length of 16,728 bp and includes a control region (loop D) of 1,270 bp containing 30 tandem repeats of a 10-nucleotyde motif between conserved sequence blocks. This is not the absolute length, due to heteroplasmy. The canine coding mtDNA consists of 22 tRNA genes, 2 rRNA genes, and 13 protein-encoding genes. The organization and arrangement of genes, as well as the use of codons, are the same as in other mammals (3, 21).

There are many research publications in which changes in the human mitochondrial genome associated with the development of cancer diseases are analyzed. Owing to the high degree of similarity in the structure of the mtDNA of *Homo sapiens* and that of *Canis lupus* familaris, it could be expected that specific changes in specific genes of the two species may result in similar disorders in the course of basic cellular processes and contribute to malignant transformation. Table 2 shows only some most frequently explored changes in the human mtDNA in cancer diseases. It should be emphasized, however, that, in principle, mutations and tumor-related polymorphisms occur in all mtDNA-coding fragments (16).

Earlier studies were focused primarily on human mtDNA mutations, and only a few authors performed a detailed analysis of the effect of mutations on the process of neoplasia in dogs (3, 29).

The most variable and the best known region in the mitochondrial genome in man is the D-loop (displacement loop). Mutations localized in the D-loop may affect the replication and transcription of mtDNA. In 19 cases of female mammary cancer one heteroplasmic change was shown, while the others were homoplasmic. Heteroplasmy involved the D-loop region (45). The most frequent mutations (38%) in breast cancer involve the D-loop region. Among the 45 mutations described, 11 resulted in the conversion of the amino acid in the encoded protein. The mutations involved NADH dehydrogenase subunit 2, ATPases of subunit 8, COIII, and cytochrome b (52).

Bertagnolli et al. (3) investigated mixed canine mammary tumors and performed an analysis of a fragment of the D-loop of mtDNA from two tumor components (epithelial and mesenchymal). A few cases of base substitution, mostly heteroplasmic, were demonstrated. Heteroplasmic changes were found in four positions, mainly in epithelial tumors. The total mutation rate was not high, and in most cases, individual tumor samples contained mtDNA with similar haplotypes, which suggests that the epithelial and mesenchymal elements have a common origin.

As one of molecular markers, the D loop sequence was also used to determine the clonal origin and evolution of canine transmissible venereal tumor from different continents (29). A large number of mutations and heteroplasmy were identified, and each case was was found to be genetically distant from the host cells, from which it was derived. It was shown that cancers in 40 dogs from 5 continents were derived from a single neoplastic clone, which had divided into two subclones during evolution (29).

Parrella et al. (31) demonstrated the presence of a substitution in the CYTB and ND1 gene sequences derived from patients diagnosed with breast cancer. Dasgupta et al. (7) proved the effect of the overexpression of a 21-bp deletion mutation of CYTB in bladder cancer in humans and mouse models. An increased growth of the tumor was accompanied by the Warburg effect (7). In other studies (48), the identified CYTB mutant was regarded as a strong antigen in melanoma patients.

The analysis of mutations in the mitochondrial genes in human bladder, head and neck, and lung primary tumors (12) revealed a high frequency of mitochondrial DNA (mtDNA) mutations. The majority of these mutations were homoplasmic, which suggests that mutant mtDNA became dominant in tumor cells (Tab. 2).

Prostate cancer is a tumor, whose development is related to mutations in the subunit I of complex IV (COI) (32). It was shown that 11-12% of patients had mutations within COI, which changed the conserved amino acid sequence in the encoded protein. In three patients, heteroplasmic changes were detected, one of which led to the formation of a stop codon (32).

So far, no mtDNA mutation characteristic of a given type of cancer has been detected. After many years of investigations of mtDNA mutations in malignant trans-

Tab. 2. Selected mtDNA regions whose mutations predispose to human and canine cancer

Gene /region	Predisposition to cancer	
	Human	Dog
D-loop	breast cancer, bladder tumors, head and neck cancer, lung cancer (12, 45, 52)	breast cancer, canine transmissible venereal tumor (3, 29)
tRNA genes	breast cancer (17)	no data
NADH (dehydrogenase subunit 2,3,4 (ND2, ND3, ND4) gene	bladder tumors, head and neck tumors, breast cancer (12, 52)	no data
CYTB (cytochrome b)	bladder tumors, breast cancer, melanoma (7, 12, 31, 48, 52)	no data
COI, COIII (cytochrome oxidase subunit I, III)	prostate cancer, breast cancer (32, 52)	no data

formation, it still remains unexplained whether mutations present in mitochondrial DNA are primary in relation to cancer or secondary, arising in the course of neoplasia. The fact that mutations are manifested a long time after they take place (at the time of the dominance of mutant DNA), as well as the slowly proceeding character of the symptom, indicates their primary character. Despite intensive research, the impact of mitochondria on cancerogenesis still remains unexplained.

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