Research Article

Features of the Expression of Some Interferon- and Tumour-Suppressor Genes in Cats with Benign and Malignant Neoplasms of the Mammary gland

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Abstract | **Background.** An assessing the expression of tumour-suppressor genes is a diagnostic approach, included in the practice of veterinary oncologists. Complementing these laboratory tests with methods for assessing the expression of interferon (IFN) genes can expand the possibilities of diagnosing animal cancers and improve the accuracy of differential diagnosis of benign and malignant neoplasms, in particular of the mammary gland (MG). **Methods**. Surgical material obtained after unilateral mastectomy with simultaneous hysterovariectomy, as well as peripheral blood samples of cats with MG tumours was used. Blood samples and surgical material obtained from healthy cats after a planned hysterovariectomy were used as control. In cats with established diagnosis of stage II breast cancer (BC; n = 4), fibrocystic mastopathy (FCM; n = 3) and healthy cats (n = 4), the following were examined: (1) haematological parameters; (2) biochemical blood indicators; (3) expression of *ifnα7, ifnβ1, ifnγ, ifnλ1, p53, rb1, cdkn2a* and *gadd45g* genes in the BC and FCM tumor tissues, as well in the uterus tissue and WBCs using qPCR. **Results**. In cats with MG tumours, signs of lymphopenia and thrombocytopenia were found, these being especially pronounced in animals with BC. In cats with BC and FCM, there were significant differences in the expression patterns of the spectrum of target IFN system genes and a number of key cell cycle control factors. These differences were expressed in the tumour tissue itself, in WBCs and in the uterus tissue. Decreases of *gadd45g* and *ifnλ1* genes expression in BC tumour tissue were the most significant. **Recent findings and conclusions.** The high level of *p53, rb1* and *cdkn2a* gene expression in animals with BC can serve as a criterion for the intensity of the mechanisms of antitumour surveillance in general. However due to the inhibition of *gadd45g* gene expression, the described expression pattern indicates an imbalance in the molecular censor systems that protect the health of the cellular genome. Evaluation of the expression levels of this spectrum of genes, especially the *gadd45g* and *ifnλ1* genes, may be useful as an additional criterion for the differential diagnosis of benign and malignant breast diseases in cats.

Keywords | Breast cancer, Mastopathy, Interferon genes, Tumour-suppressor genes, Expression

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INTRODUCTION

Laboratory methods for assessing the expression of oncogenes and tumour suppressors are currently increasingly in demand in the practices of veterinarians working with oncological patients. Despite this diagnostic approach long being popular in clinical practice, it does not always provide unambiguous information. For example, the absence has been described of mRNA of the *c-kit* gene in mesothelioma tissue samples, despite the fact that these tissues are characterised by a strong specific immune histochemical staining for this receptor (Horvai et al., 2003). However, in most other cases the use of such diagnostic methods is characterised by high resolution. For example, a significant decrease has been described in the mRNA content of the *p53* and *bcl-2* genes in the cells of breast cancer (BC) metastases in the brain compared to in the cells of the primary tumour. Moreover, these results correlate with the data of immunohistochemical staining (Stark et al., 2006).

Practitioners in the local veterinary clinic use such molecular markers as CK-Pan (pan Cytokeratin), p40 and S100 for the differential diagnosis of oncological diseases of different histogenesis. Diagnostic methods for assessing the expression of the TP63 protein are also used. This is a transcription factor from the *p53* gene family, which also includes the *p73* gene. The products of these genes are now regarded as essential for the control of cell differentiation, proliferation and survival in various tissues. Therefore, the study of the expression of this gene family is recommended to differentiate tumours by their histogenesis (Gatti et al., 2019; Steurer et al., 2021).

Testing the expression level of such genes may be considered as one of the methods for assessing the degree of a neoplasm malignancy (Graziano & De Laurenzi, 2011; Steurer et al., 2021) as well as a criterion for choosing the complex therapy methods of an oncological disease and evaluating the effectiveness of such therapies (Borrero & El-Deiry, 2021).

The prognostic value of assessing the expression of cell cycle control genes (*p53* and *gadd45g*), as well as some genes of the interferon (IFN) system (*ifnα7, ifnβ1, ifnγ* and *ifnλ1*) in haemoblastosis of cats to determine the risk of the disease progression to a more aggressive stage has been confirmed in our previous studies (Tsybulsky et al., 2022).

An evaluation of the feasibility of such a diagnostic approach in relation to animals with tumour neoplasms of MG of different histogenesis and different degrees of malignancy was made in the present work. In addition to the above list of molecular biological tests, we examined the expression of important tumour suppressors such as *rb1* and *cdkn2a* (cyclin-dependent kinase inhibitor 2A) in animals with MG tumours. The *rb1* gene encodes the retinoblastoma protein (pRb), which is one of the most important antitumour suppressors. In the genome of cats, this gene has a size of 181,640 bp and consists of 30 exons. Eight transcripts have been described for this gene (Gene ID: 101101356, updated 4 Jan 2022). The presence of several alternative splicing products creates significant difficulties in assessing the correlation of this gene expression level with the pathogenesis of cancer. Nevertheless, given the exceptional importance of the products of *rb1*in the control of normal and pathologically altered cell cycles, the development of a methodology for assessing this parameter in veterinary practice is considered necessary. It is important to note that the disruption of the normal expression of the pRb protein has a high level of penetrance, with many types of oncological pathologies in humans and animals due to disorganisation of the cell cycle checkpoint at the level of CDK4/6 kinase activity and E2F-DP transcription factors (Chakraborty et al., 2021). Changes in the expression of the pRb protein also affect the immunological characteristics of a tumour and its microenvironment; therefore, the assessment of the expression of this tumour suppressor may be useful in predicting the dynamics of the disease and evaluating the effectiveness of a therapy (Knudsen et al., 2019).

Estimation of the *cdkn2a* expression is a non-trivial task. Modulation of this gene expression by alternative splicing leads to the appearance of two transcripts, the products of which differ greatly in structure; however, all of them exhibit a wide range of regulatory functions in relation to the mechanisms of antitumour surveillance. In particular, the $p16^{INK4A}$ (*p16*) transcript product is a tumour suppressor that modulates the cell cycle at the level of the pRb-regulated G1-to-S phase transition, inhibiting the kinase activity of CDK4 and CDK6 (Palmieri et al., 2009; Witkiewicz et al., 2011). Of no less interest is the $p14^{ARF}$ transcript variant, the product of which is an important factor for the stabilisation and protection of the TP53 protein from ubiquitin-dependent proteolysis. The product of $p14^{ARF}$ is a physiological inhibitor of MDM2 (an E3 ubiquitin ligase that controls P53 activity and stability). Loss of $P14^{ARF}$ activity may have the same effect as the loss of P53 function (Agrawal et al., 2006). However, the $P14^{ARF}$ transcript has not been described in cats. It is also impossible to assume its presence by analogy with human $P14^{ARF}$ due to the fact that during the formation of the $p14^{ARF}$ transcript in humans a region from outside the gene is involved. Therefore, in this study we evaluated only the expression of the $p16^{INK4A}$ transcript.

MATERIALS AND METHODS

Animals

Domestic non-pedigree cats with a neoplasm of the MG detected for the first time during examination in the veterinary clinic. The age of cats was 7–15 years. All animals underwent the surgical treatment of unilateral mastectomy with simultaneous hysterovariectomy. According to the results of histological examination, two groups of animals were formed: (1) cats diagnosed with BC stage II (n = 4) and (2) cats with FCM (n = 3).

The healthy cats (n = 4) operated on for planned sterilisation were used as control animals. The age of the control cats was 4–7 years.

Ethical standards

All manipulations with animals were carried out in accordance with the regulations approved for veterinary clinics in Russia.

Biological material

Blood samples and operational material of the tissue of the MG and uterus were taken. The peripheral blood was taken in a volume of 2 mL from the lateral vein of the forelimb.

Haematological analysis was performed using a Cell Dyn 3700 haematological analyser (Abbott, USA).

Biochemical analysis of blood was performed using a URIT-800 VET biochemical analyser (URIT Medical Electronic Co., Ltd., China).

Histological examination of a macropreparation of operational material of MG with a tumour was performed in accordance with the diagnostic algorithms recommended for the histology of breast and soft-tissue tumours (Goldschmidt et al., 2017; Hendrick, 2017). Histological preparations were stained with haematoxylin-eosin according to the methods of Pappenheim and Papanicolaou.

Analysis of target gene expression: Extraction of the total RNA from the cats' white blood cells (WBCs) was performed with the ExtractRNA reagent (Eurogen, Russia) according to the manufacturer's recommendations. The concentration and quality of the isolated RNA were analysed with a NanoDrop 2000C spectrophotometer (Thermo Scientific, USA). Reverse transcription was performed using the MMLV RT reagent kit (Eurogen) with the Oligo(dT) primer. All procedures were performed according to the manufacturer's recommendations.

The expression of the following feline genes was analysed: (1) IFN genes: *ifnα7, ifnβ1, ifnγ* and *ifnλ1;* (2) tumour-suppressor genes: *rb1, p53, cdkn2a* and *gadd45g*; and (3) *gapdh* (glyceraldehyde 3-phosphate dehydrogenase) as housekeeping gene.

Primer-BLAST NCBI and SnapGene 4.1.8 were used to design the primers. The following pairs of forward (F) and reverse (R) gene-specific primers were used (Table 1).

Gene expression analysis was carried out using the qPCR mix HS SYBR (Eurogen) by qPCR on a CFX96 thermocycler (Bio-Rad, USA). The *gapdh* gene was used as a normaliser. Samples of cDNA derived from the mRNA of healthy cats were used as control. Relative differences in the expression parameters of each gene in the group of animals with stage II breast BC and FCM in comparison with the control group were obtained by calculating the multiplicity of expression variation (fold) according to a previously described method (Livak and Schmittgen, 2001). The expression index of the target gene in the control group was taken as a conditional 1 unit.

Statistical analysis

Statistical analysis of the data was carried out using the SPSS 11.0 statistical analysis software with the determination of the nonparametric Mann–Whitney U-test. Differences were considered significant if the significance level (a) was <0.05.

RESULTS

The general condition of the cats examined was characterised as relatively satisfactory. According to the results of the preoperative blood test, there were no significant changes in the cellular composition or biochemical parameters of the blood. All studied parameters were within the ranges typical for healthy cats, as adopted in the laboratory of the veterinary clinic. However, some blood parameters of animals with MG neoplasms differed significantly from those of healthy control animals (Table 2).

Especially significant were the changes in the blood formula, characterising the absolute and relative content of lymphocytes. In cats with breast neoplasms, the relative content of lymphocytes was significantly (a < 0.05) lower than in control animals. At the same time, in cats with BC such a significant decrease in the absolute content of lymphocytes suggests the significance of lymphopenia in the pathogenesis of cancer. Elevated levels of granulocytes and erythrocyte sedimentation rate (ESR) against the background of lymphopenia indicate an inflammatory process in the body of tumour-bearing animals. This process is predominantly non-specific. In animals with BC, a decrease in blood platelets was also determined, which apparently reflects the general toxic nature of the pathological process.

Table 1: Primer sequences specific for feline target genes

Feline Gene	Forvard primer (5`-3`)	Reverse primer (5`-3`)	Product length: bp
ifnα7	CCTGACGAACGAGGACATTCA	TGGAAAGTGTGGTGTGATGAG	237
ifnβ1	CTCCACTGGCAGAAGGAACA	AAGGGTCGTATTGTCCCAGG	84
ifnγ	ATCCAGATGTAGCAGATGGTGGG	TCCATGCTCCTTTGAATGCG	160
ifnλ1	TGCCTTGGAAAATTCGCTGA	CAGCCTTGAGACTCCTTCCT	321
gadd45g	ATCGACATCGTGCGCGTGGG	TCGTTGACACTGCGGCTCTCC	182
p53	GAGGTCGGCTCTGACTGTACCAC	GCTCGCTTAGTGCTCCCAGG	251
*rb1**	CAAAGGACCGAGAAGGACCAG	GAAGGCTGAGGTTGCTTGTGC	179
cdkn2a	TGGACACGCTGGTGGTGCTG	CGGCTGTCTGCGACACCTTC	193
Gapdh	TGACCCCTTCATTGACCTCA	TTCACGCCCATCACAAACAT	306

*- The *rb1* gene in F. catus consists of 30 exons. The eight transcripts are known for this gene. Primers for assessing the level of expression of this gene were developed taking into account differences in

Table 2: Results of haematological and blood biochemistry analyses of cats with MG tumours and healthy cats

	Stage II breast cancer (8–11 years old)	Fibrocystic mastopathy (7–15 years old)	Control (healthy cats) (4–7 years old)	Reference values of healthy cats [2]
Number of animals	n = 4	n = 3	n = 4	n = 2856
Haematological Parameters				
WBC × 10^9/L	6.2 ± 1.9	8.3 ± 1.2	7.9 ± 2.4	5.5–19.5
Lymphocytes, × 10^9/L	1.3 ± 0.4*[1]	2.5 ± 0.4	2.8 ± 0.5	0.8–7
Monocytes, × 10^9/L	0.4 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0–1.9
Granulocytes, × 10^9/L	4.5 ± 0.8	5.6 ± 0.5	4.6 ± 1.3	2.1–15
Lymphocytes, %	19.4 ± 1.9*[1]	29.5 ± 1.4*	37.5 ± 2.1	12–45
Monocytes, %	7.8 ± 1.1* [1]	3.3 ± 0.6	4.2 ± 0.7	2–9
Granulocytes, %	72.8 ± 2.9*	67.2 ± 1.8	58.2 ± 2.2	35–85
RBC, × 10^{12}/L	5.3 ± 1.3	4.8 ± 0.5	5.6 ± 0.8	4.6–10.0
HGB, g/L	92 ± 6*	122 ± 8	143 ± 14	93–153
HCT, %	27.3 ± 2.4*	31.5 ± 0.9	34.6 ± 1.2	28–49
PLT, × 10^9/L	223 ± 39* [1]	394 ± 28	404 ± 37	100–514
PCT, %	0.27 ± 0.09	0.19 ± 0.07	0.24 ± 0.06	0.11–0.28
Eosinophils, %	7.9 ± 1.2* [1]	3.4 ± 1.5	2.6 ± 1.4	2–7
ESR, mm/h	44 ± 8*	29 ± 6*	9 ± 3	3–10
Indicators of Blood Biochemistry				
Creatinine, mmol/L	164.4 ± 12.6*	128.6 ± 19.5	92 ± 13	71–159
Urea mmol/L	9.7 ± 1.8*	7.5 ± 2.3	4.3 ± 1.2	3.6–7.1
Glucose, mmol/L	4.2 ± 0.5	4.3 ± 0.8	4.5 ± 0.6	3–6

Notes: *– differences with the control group are significant (a < 0.05)

[1] – differences with the group of animals with fibrocystic mastopathy are significant (a < 0.05)

[2] – reference indicators of healthy cats according to the archive of the veterinary clinic (2010–2021).

cess. This toxic component of the cancer pathogenesis was much more pronounced in animals with BC than in those with FCM.

Analysis of the blood biochemical parameters in animals with BC allowed establishing a significant increase of creatinine and blood urea in comparison with the control. To a certain extent, this may be associated with the development of the so-called paraneoplastic syndrome.

The histology of breast preparations with a tumour turned out to be quite diverse: from adenocarcinoma and metaplastic carcinoma to tumours with a predominance of connective tissue elements such as a moderately differentiated fibrosarcoma. However, there were some common features of the histological picture: 1) a poorly demarcated, non-encapsulated, monophasic, moderate cell tumour was visualised, characterised by a locally invasive type of growth; 2) the tumour contained spindle-shaped cells with indistinct

intercellular boundaries, weakly eosinophilic cytoplasm and ovoid nuclei with finely dispersed chromatin and small basophilic nucleoli; 3) the degree of atypia was moderate. Mitosis figures, including pathological ones, were visualised in tumour cells. Cells formed long inter-anastomosing bundles. The tumour stroma was connective tissue, moderately vascularised by thin-walled and thick-walled rounded vessels, with moderate focal lymphocytic infiltration along the periphery of tumour growth and extensive areactive necrosis (up to 50% of the tumour area). In the superficial sections, the tumour spread to the superficial layer of the dermis.

The presence of pronounced phenomena of tumour necrotisation is obviously the cause of the above-described phenomena of haemo- and general toxicity in animals with BC. We have to admit that determination by histological criteria of the group of animals with BC is not strictly standardised. However, the clinical symptoms and haematological parameters in these animals turned out to be quite similar and differed markedly from the corresponding indicators of healthy control animals, as well as from those of cats with benign breast pathology (especially the content of lymphoid blood cells).

Significant differences in the examined groups were found in the expression of several genes of the IFN system, as well as tumour-suppressor genes. The samples were taken from tumour tissue, blood and uterus of cats with breast neoplasms.

Significant increases in the expression of *ifnα7, ifnβ1,* and *ifnγ* genes in animals with BC (by 15-, 9- and 5-fold, respectively) were found (Figure 1A.).

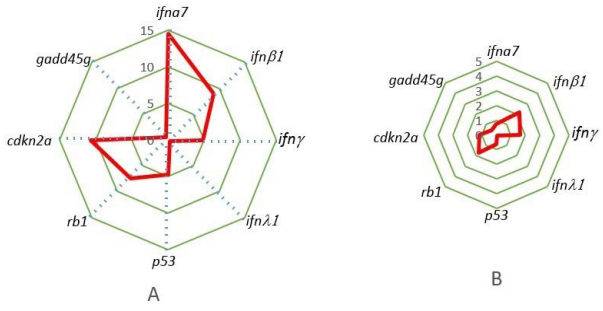

Figure 1: Expression of some IFN system and tumour-suppressor genes in feline MG neoplasm tissues: (A) BC; (B) FCM. Changes in the concentration of mRNA of the corresponding genes relative to the indicators of cats in the control group, taken as 1 unit, are presented.

The transcriptome of the tumour tissue of cats with BC was also characterised by statistically significant ($a < 0.05$) activation of gene expression of important antitumour protection factors such as p53, cdkn2a and rb1. However, the expression of the *gadd45g* gene (an important factor in the regulation of the cell cycle and a marker of the cellular genome health) was inhibited to a level only 0.46 of that observed in healthy animals of the control group. A decrease in the activity of GADD45G can have (naturally, along with other reasons) far-reaching consequences in the form of inefficiency of cell cycle arrest molecular mechanisms, induction of DNA repair mechanisms and apoptosis.

The analysed range of parameters in the benign neoplasm tissue of the breast was radically different (Figure 1B). Quantitative indicators of the expression activity of the studied genes were insignificant. Expressions of *ifnα7, p53* and *gadd45g* were markedly reduced, amounting to 0.75, 0.61 and 0.43, respectively, of the indicators of control animals.

Type III IFNs are specialised for antiviral and antitumour protection of epithelial tissues. The tumour tissue transcriptome of both groups of cats with BC and FCM was characterised by inhibition of *ifnλ1* gene expression (0.46 and 0.43, respectively, compared to healthy animals). This observation reflects the insufficient reactivity level of the connective and epithelial tissue in which the tumour process develops. The assessment of this parameter may be important in relation to the prognosis of cancer development. The expression of the same spectrum of genes was also analysed in the WBCs of cats with BC and FCM, as well as in healthy control animals (Figure 2).

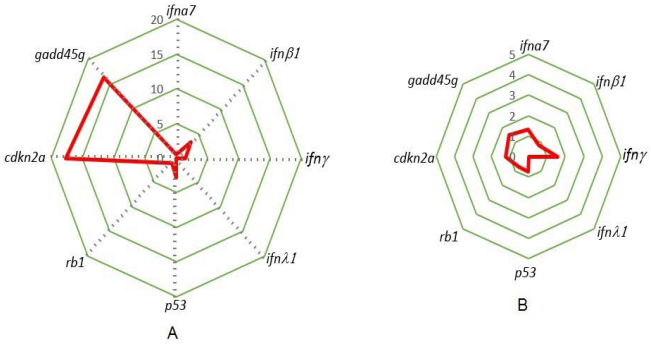

Figure 2: Expression of some IFN system and tumour-suppressor genes in the WBCs of cats with MG neoplasm: (A) BC; (B) FCM. Changes in the concentration of mRNA of the corresponding genes relative to the indicators of cats in the control group, taken as 1 unit, are presented.

In contrast to the tumour tissue, a significant (16-fold) activation of the *gadd45g* gene expression was found in the blood leukocytes of cats with BC (Figure 2A). There was also a 17-fold increase in the level of *cdkn2a* gene expression, along with 2.9-fold activation of *p53* gene expression in comparison to healthy animals.

Expression of the IFN system genes in blood leukocytes

of cats with BC (Figure 2A) was much less pronounced compared to the BC tumour tissue of the same animals (Figure 1A). Gene expressions of *ifnβ1* and *ifnγ* were activated in the WBCs of cats with BC, by 3.3- and 1.5-fold, respectively, compared to the control. At the same time, *ifnα7* gene expression was practically unchanged, and *ifnλ1* expression was completely absent in the WBCs of cats with BC.

In animals with FCM, the expression of the described spectrum of genes in WBCs practically coincided with those of healthy animals. Only the expression of *ifnγ*, the level of which was slightly (1.6-fold) increased, was the exception (Figure 2B). The increased activity of *ifnγ* is apparently associated with a chronic inflammatory process in the tissues of the MG and reproductive organs affected by the fibrocystic pathological process.

During the examination of the surgical material obtained from ovario-hysterectomy, we found that all cats with FCM had comorbidities, with pronounced fibrocystic degeneration of the uterus and ovaries. In cats with BC, such changes in the tissues of the reproductive organs were expressed to a much lesser extent and were absent in healthy cats. In all three groups of examined animals, we studied the transcriptome of the uterine tissue. Significant differences were found in target gene expression between animals with BC and FCM (Figure 3).

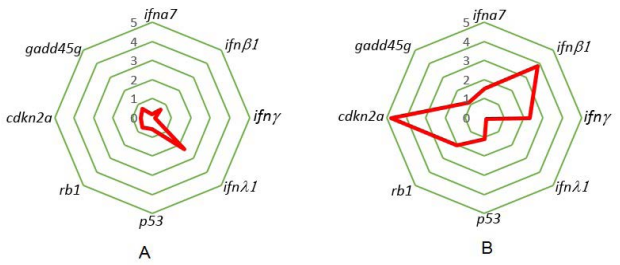

Figure 3: Expression of some IFN system and tumour-suppressor genes in the uterine tissues of cats with MG neoplasm: (A) BC; (B) FCM. Changes in the concentration of mRNA of the corresponding genes relative to the indicators of cats in the control group, taken as 1 unit, are presented.

In the transcriptome of the uterine tissue of cats with BC, a noticeable (approximately 50%) inhibition of the expression of all the studied genes was found, with the exception of the *ifnλ1* gene, the expression of which was 2.3-fold higher than in the animals of the control group (Fig. 3A). At the same time, a fundamentally different expression pattern was established in the group of animals with FCM (Fig. 3B). There was a 3.8-fold increase in *ifnβ1* expression, a 2.3-fold increase in *ifnγ*) and a 2.3-fold increase in *rb1* gene expression. The expression of the *p53* and *ifnα7* genes was at the level of the corresponding indicators of healthy control animals. However, in this case, a profound suppression of *ifnλ1* gene expression was found.

We assess such an expression pattern of the studied genes in the uterine tissue of cats with FCM as corresponding to the state of intense functioning of antitumour surveillance mechanisms that counteract malignant transformation of pathologically altered tissue. At the same time, these mechanisms are unable effectively to stop the chronic inflammatory process that occurs in the tissues of the reproductive organs and which ultimately leads to the formation of large-scale metaplasia, such as fibrocystic degeneration.

DISCUSSION

In the present study, cats with FCM-type benign breast neoplasms, as well as those with stage II BC were examined. All cats had a preliminary diagnosis of BC in the operable stage of the oncological process. In all cases, the same type of surgical treatment, in the form of a unilateral mastectomy with simultaneous hysterovariectomy, was performed. The final diagnosis was made according to histological examination of the surgical material. In accordance with this, the operated animals were divided into two groups: (1) stage II BC, and (2) FCM.

Some significant differences in laboratory parameters between animals with BC and with FCM were identified.

In general, changes in hematological parameters were quite typical, reflecting the presence of a chronic inflammatory process, which usually develops in patients with tumors (Lim et al., 2018). In cats with BC and FCM, a significant increase in ESR was determined. This indicator was especially high in cats with BC, which is apparently associated with the induction of inflammatory reactions due to the formation of a large number of necrosis zones in the tumour tissue (which was shown during histological examination of the tumour tissue). The development of paraneoplastic syndrome in animals with BC is apparently associated with inhibition of thrombocytopoiesis, which is manifested by statistically significant ($a < 0.05$) thrombocytopenia, as well as increased levels of creatinine and urea in the blood. A general trend was established: all of these haematological and biochemical parameters were higher in cats with BC compared to those with FCM.

Particularly noticeable changes in the blood formula in tumour-bearing animals were found in relation to the lymphoid component of the blood. A statistically significant ($a < 0.05$) decrease in the percentage of lymphocytes was found both in animals with BC and with FCM. However, in cats with BC, the tendency to develop lymphope-

nia was much more pronounced: the absolute content of lymphocytes in the blood of these animals was more than 2-fold lower than in healthy control animals. In general, this is a fairly well-known haematological phenomenon, typical of oncological processes of different histogenesis (Menetrier-Caux et al., 2019). There was a decrease in the number and competence of the immune system lymphoid elements against the background of a predominance of granulocyte blood elements, which are participants in non-specific inflammatory processes occurring in the body of tumour-bearing patients (Ocana et al., 2017). Despite the differential diagnostic value of such criteria being relative, in combination with more targeted tests designed to assess immunological and other parameters characterising the mechanisms of cellular and molecular antitumour surveillance, the assessment of the above haematological criteria is quite applicable.

The expression pattern studied in this work is represented by genes, the expression of which turned out to be diagnostically significant in our previous work (Tsybulsky et al., 2022). To this gene spectrum in this study, we added an analysis of the *rb1* and *cdkn2a* gene expression. The products of these genes are important elements of the molecular cell cycle control systems and antitumour resistance. A deficiency in the function of these factors leads to increased resistance of tumour cells to apoptosis-inducing mechanisms (Chakraborty et al., 2021).

The expression of the IFN system and tumour-suppressor genes chosen in this study differed significantly between cats with benign and malignant breast diseases. To the greatest extent, these differences were pronounced in the breast tumour tissue itself and in WBCs.

A significant (5–15-fold) increase was found in the expression of *ifnα7, ifnβ1, ifnγ, cdkn2a, rb1* and *p53* genes in BC tumour tissue. Expression of these genes in the MG metaplastic tissue from cats with FCM was significantly lower and, in general, did not differ significantly from that of control animals. This indicates the relative inertness of these censor systems in the tumour tissue of animals with FCM.

Along with large differences in most parameters of the expression pattern, we were able to identify a common molecular criterion that is characteristic for both groups of animals with BC pathology: inhibition of the *ifnλ1* and *gadd45g* gene expression in tumour tissue (Figure 1).

The products of the *gadd45* gene are one of the most significant components of the tumour suppression system, of which the GADD45g variant attracts special attention (Tamura et al., 2012). A decrease in the expression level of this protein is associated with a wide variety of oncological diseases of different histogenesis. With regard to BC, there is evidence that the GADD45g protein inhibits the growth of human BC cells *in vivo* and *in vitro*, acting as an antitumour agent (Zhang et al., 2021). Moreover, the antitumour effect of the GADD45g protein can be realised independently of other important molecular factors that counteract carcinogenesis (P53, pRB, etc.; Zhang et al., 2014). The *ifnλ1* gene product is a type III IFN system specialised for epithelial tissues, whose role, along with protection against viral pathogens, is extremely important for maintaining the genetic stability of endothelial cells and epithelial tissues (Lasfar et al., 2016; Walker et al., 2021).

Thus, insufficient expression of the *ifnλ1* and *gadd45g* genes may indicate a reduced activity of molecular censor systems that counteract the processes of mutagenesis and carcinogenesis. We have previously found the same changes in the expression pattern in cats with lymphoblastoses. Moreover, a decrease in the expression activity of these genes is correlated with the risk of the disease transition to a more aggressive phase (Tsybulsky et al., 2021). According to our assumptions, such changes in the expression pattern may serve as a prognostic criterion for forms of oncological pathology in cats, such as BC. It can be supposed that it is advisable to correct these indicators by methods of targeted induction of the corresponding genes. Substitution therapy with appropriate recombinant products as part of the complex therapy for oncological diseases also seems to be a promising method for disease treatment. Testing these working hypotheses requires more extensive research and the development of appropriate therapeutic technologies. These questions relate to the area of our scientific interests and the genetic engineering technologies being developed. It must be noted that the development of such methods of targeted therapy should be more focused on the activation of the expression of these genes in tumour cells themselves or in immunocompetent cells infiltrating the tumour. This is due to the fact that the expression of the *gadd45g* gene is at a very high level in peripheral blood leukocytes against the background of its expression inhibition in the tumour tissue itself. With regard to the *ifnλ1* gene, the need to induce the expression of this gene *in situ* is even more clear, since peripheral WBCs are not specialised producers of this cytokine.

(Wack et al., 2015). It is difficult to explain that a noticeable expression inhibition of the entire range of genes studied (with the exception of *ifnλ1*) was found in the uterine tissue of cats with BC. Moreover, in two cats with BC the expression rates of the entire group of tumour-suppressor genes were less than 50% of the corresponding indicators in the control animals. In the uterine tissue of these an-

imals, the expression of *ifnα7* and *ifnγ* genes was practically absent. Speculatively, these data can be interpreted as evidence of the suppression of antitumour surveillance mechanisms in the reproductive system of animals with BC, as well as evidence of the absence of tissue infiltration by immunocompetent cells and, accordingly, the absence of inflammation. To a certain extent, this can also be associated with the formation of a state of lymphopenia, which is most pronounced in cats with BC.

However, all these assumptions require appropriate experimental verification. It should be noted that the features of the expression pattern presented in this report were established precisely in animals with stage II BC, i.e., at the operable stage of the disease. A separate study requires the assessment of changes in the expression of the studied spectrum of genes in animals with BC at other clinical stages.

CONCLUSIONS

The differences of the IFN and a number of key tumor supressor genes expression patterns are expressed in the tumour tissue itself, in WBCs and in the tissue of the uterus in cats with BC and FCM. We consider the suppression of *gadd45g* and *ifnλ1* gene expression in BC tumour tissue to be the most significant. The high level of *p53*, *rb1* and *cdkn2a* gene expression in animals with BC can serve as a molecular criterion for the intensity of the cell cycle control mechanisms and antitumour surveillance in general. However, the obviously insufficient level of expression of the *gadd45g* gene makes it possible to interpret such an expression pattern as a sign of an imbalance in the molecular censor systems for protecting the health of the cellular genome.

Estimation of the expression levels of *p53*, *rb1*, *cdkn2a*, *gadd45g* and *ifnλ1* genes may be useful in the differential diagnosis of benign and malignant breast diseases in cats. Therapy based on the targeted modulation of *gadd45g* and *ifnλ1* gene expression may be a useful addition to the complex therapy of cats with breast cancer.

Ethics approval and consent to participate

This research was approved by ethical standards governing the work of veterinary clinics in Vladivostok and comprised a study of samples collected with full informed consent from owners who agreed to the use of such samples for research purposes.

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CONFLICT OF INTEREST

No competing financial interests exist.

AUTHORS' CONTRIBUTIONS

Each author made substantial contributions to the conception and design of the work and the acquisition and analysis of blood and DNA/RNA samples. The first author performed the final analysis of all results and prepared the final version of this original article. All authors read and approved the final manuscript.

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