# Proliferative activity of normal tissues and malignant tumors during infestation of the organism by a biotic ecopathogen

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**Abstract.** The article shows the role of metabolites of metacercariae and opisthorchiae in the induction of proliferative activity of somatic cells of the hepatobiliary system and other organs. The importance of cellular and tissue bioregulators in increasing the level of proliferative activity of various tissues in organisms infested by the trematode *Opisthorchis felineus* is considered. It follows from the data obtained that the biological function of a tissue-specific keilon–containing effector produced by differentiated cells and inhibiting cell proliferation based on the biological feedback principle, in comparison with the background of prolonged parasitization of this ecopathogenic helminth in the hepatobiliary system of the body, decreases. There is also a decrease in the sensitivity of somatic cells to a tissue-specific growth inhibiting factor. It follows that the activation of cellular and tissue proliferation of animals long-term infested by opisthorchiasis is also due to a violation of the mechanisms of regulation of cellular and tissue homeostasis. This is confirmed by an increase in the growth rate of syngenic malignant tumors, as well as a modification of the effect of homologous leukocyte interferon from an inhibitor of the malignant process to an activator.

## 1 Introduction

Literature analyses on the role of the natural environmental factor of the Tyumen region - opisthorchiasis infestation in the development of primary cholangiocellular liver cancer are numerous. They are confirmed by studies [1-6]. Based on the hyperendemicity of this territory for parasitization of the biotic pathogen - the helminth Opisthorchis felineus - in the hepatobiliary system of the human body, chronic opisthorchiasis is considered as a facultative precancer of the liver [4]. However, the mechanisms of promoter action of chronic infestation of the trematode Opisthorchis felineus on cholangiocarcinogenesis are poorly understood.

The basis for the emergence of many pathological processes in the body, including malignant processes, is the disruption of regulation of somatic cell reproduction, i.e. tissue homeostasis. In this case, an environment determining the proliferative activity of somatic cells and their transformation is formed [3, 7]. It has been established that chronic opisthorchiasis infestation of the organism by trematode Opisthorhis felineus is

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accompanied by active proliferation of bile duct cells, pericholangiofibrosis, metaplasia and adenomatosis of ductal epithelium [1-9 ].

Regulation of cell proliferation intensity is mediated by mediators synthesized by somatic cells of various tissues, including malignant ones. One of such mediators is tissue factor (keilons) produced by differentiated cells and inhibiting cell proliferation on the basis of the biological feedback principle. It possesses tissue specificity [10-11].

The fact that opisthorchiasis infestation induces activation of cell proliferation processes in the tissues of the organism gives reason to believe that the mechanisms of regulation of tissue homeostasis, including growth-inhibiting tissue-specific keilon factor, are disturbed. However, the mentioned mechanisms of regulation of cell-tissue proliferation in opisthorchiasis infestation are insufficiently studied; such data are almost not found in scientific literature. In this connection, *the aim of the experimental study* was to investigate the processes of tissue proliferation, inhibitory activity of exogenous tissue-specific membrane-tropic keilon-containing factor and sensitivity of somatic cells to it during infestation of the organism by the helminth *Opisthorchis felineus*.

## 2 Materials and methods

The research work was carried out in the Laboratory of Carcinogenesis of the Oncology Department of the Tyumen State Medical University of the Ministry of Health of the Russian Federation. Mice of inbred lines F1 [CBA/Lac x C57B1/6], CBA/Lac, DBA/2, Balb/c, A/sn (Y) were used as research objects to study the effect of opisthorchiasis infestation on the body's homeostasis regulation systems. The objects of the study obtained from the collection of the N.N. Blokhin National Research Institute of Oncology of the Ministry of Health of the Russian Federation are syngeneic in vivo transferable tumor strains: cervical cancer -5, colon carcinoma (ACATOL).

In order to exclude the mechanical pressure of helminths on the ductal epithelium and to study in vivo the biological effects of the actual metabolites of metacercariae and opisthorchiasis on cholangiocytes, hepatocytes and other somatic cells, a model was chosen for remote testing of the effect of metabolites on body tissues by culturing mature larvae and morites in intraperitoneal diffusion chambers using inbred mice (Y).

The metacercariae isolated from fish by the standard method were injected into the oral cavity of mice using and eye pipette (25-30 units in 0.9% NaCl). The infestation of animals by opisthorchiasis was validated by the presence of helminths (adults) in the hepatobiliary tract [12].

The proliferative activity of somatic cells in all experiments was studied by the inclusion of $H^3$-thymidine in the DNA of somatic cells and radiometry in a liquid scintillator (β-counter, pulse/sec.), as well as using autoradiography (index of labeled nuclei, NAME). In experiments, 1 hour before euthanasia, mice were injected with $H^3$-thymidine in vivo. Tissues of isolated animal organs for autoradiography were fixed in a 10.0% solution of neutral formalin.

To study its biological effect on somatic cells, a cellular-tissue bioregulator (keilon factor) was extracted from an aqueous homogenate of organ tissues of inbred mice by precipitation of glycoprotein fractions with 70-81% ethanol. The isolated fractions were lyophilized. The lyophilized extract was used for biological testing of the activity level of tissue cells and the sensitivity of somatic cells to them. Studies were performed on inbred animals with partial liver resection.

The mice were euthanized by cervical dislocation. Standard methods were used for statistical calculations. The reliability of the results (p) was evaluated according to the Student's t – criterion.

## 3 Results of the study and their discussion

As a result of experimental studies on inbred mice using metacercariae and opisthorchis culture in diffusion chambers in vivo, it was found that the increase of the proliferative activity in somatic cells of the hepatobiliary system (hepatocytes, cholangiocytes) and other organs (kidneys, pancreas, spleen, lymph nodes, etc.) is induced by metabolites of metacercariae and, subsequently, opisthorchis [12].

Also, the data obtained during a 2-month infestation of F1 [CBA/Lac x C57B1/6] mice with the ecopathogenic parasite *Opisthorchis felineus* showed a significant increase in the proliferative activity of the tissues of the following organs: liver, by 36.0%; kidney, by 42.3%; stomach, by 88.5%; pancreas, by 32.9%; and spleen, by 26.3%. Results with a similar trend were also obtained in mice of the ♂ line CBA/Lac.

With prolonged parasitization of opisthorchiasis in the hepatobiliary system of CBA/Lac mice (4 months), it was found that the proliferative activity of the choledochus epithelium, according to the results of the obtained mitotic index increased by 41.2% (2±0.01 in comparison with 0.85± 0.01; ‹0.001), and the epithelium of the intrahepatic ducts - by 45% (mitotic index 4.0±0.01 in comparison with 1.8± 0.02; ‹0.001) compared to the control. Similar data were obtained in mice of the ♂ TWO line/2. The proliferative of cholangiocytes at the time of opisthorchiasis infestation of 4 months exceeded the control by 50.0% (5.0± 0.01 in comparison with 2.5±0.01; p ‹0.001).

As for hepatocytes, their proliferative activity in different parts of hepatic lobules at the above-mentioned period of parasitic infestation is not the same. Studies performed on ♂TWO/2 with 4-month opisthorchiasis invasion showed that in the central part of the hepatic lobule, the NAME (index of labeled nuclei) compared with the control, it was reduced by 33.8% (52,6±0,8 in comparison with 79,4±1,1; p‹0,001), whereas in the middle of the liver lobule this indicator exceeds the control by 48.3% (95,8±0,7 in comparison with 64,6±0,7; p‹0,001). On the periphery of the hepatic lobule, the level of NAME also significantly prevails compared to the control group by 10.7% (62,3±0,8 in comparison with 56,6+0,7; p‹0,01). This difference is probably due to the degree of trophic supply depending on the localization of cells in the hepatic lobule. On average, the proliferative of hepatocytes (mitotic index) in opisthorchis-infected ♂ TWO/2 mice at 4 months was 23.8% higher than controls.

In addition, compared to the activation of tissue proliferation processes in most organs of inbred animals in comparison with the background of chronic opisthorchiasis infestation, the proliferative activity of bone marrow cells is constantly depressed, on average by 15%.

It is known that an increase in the proliferation activity of cellular and tissue structures of the body creates conditions for the transformation of normal cells into malignant ones. A prerequisite for the process of cell transformation and the development of carcinomas are mutations of proliferation control genes – "suppressor genes", which cause an uncontrolled increase in the production of mitogenic factors [13].

On the one hand, a decrease in the activity of the DNA reparative system is possible in the case of mutation of genes that are responsible for the function of this system. On the other hand, the loss of the ability of somatic cells to restore their own DNA structure can lead to mutations in both oncogenes and suppressor genes (anti-oncogenes), whose function in relation to the regulation of cell proliferation (inhibition) and differentiation is lost [13]. Such somatic cells stop responding to signals from the environment, they have auto- and paracrine stimulation of proliferation signals, the process of apoptosis is inhibited, as a result, genetic instability causes their transformation [14].

It should be noted that acceleration of tissue proliferation processes can be mediated by various factors. A number of authors have identified many (within 40) excretory-secretory

products of the helminth Opisthorchis felineus [15], which have properties of biological effect on host tissues.

Cell reproduction is regulated by homeostatic mechanisms of the organism at different levels: tissue (including keilon effectors), interstitial (genotropic activators) and organismal (nervous, endocrine and immunological regulation), which determine the activation or inhibition of the mitotic cycle. Obtaining data on the mechanisms of action of cell-tissue bioregulators is the basis for understanding the essence of the processes carried out in living systems. Proceeding from the fact that the system of cell cycle regulation is based on two types of signals: one of them - about genome intactness, the other - about the influence of external factors stimulating or inhibiting mitosis [18], the synthesis of keilon-containing factor by differentiated tissue cells plays a significant role in tissue regulation of the proliferation process and maintenance of structural homeostasis at the cell-tissue level [16-18].

Keilons inhibit the proliferation of immature cells of the same tissue based on a biological feedback mechanism: a decrease in the number of differentiated cells in the tissue mediates a decrease in the level of production of keilons; due to a decrease in the content of this tissue-specific effector, the mitotic activity of tissue cells increases [10-11, 17, 19]. As a result of this mechanism, the required number of cells in the tissues is maintained. It follows that the study of the mechanism of regulation of tissue homeostasis in opisthorchiasis infestation of the body by a keilon-containing factor is of great importance for determining its role in the promotion of cholangiocanceogenesis [20].

Due to the fact that in inbred mice it is not possible to isolate tissues lining bile ducts and obtain keilon-containing extract from them, the data obtained on the model of hepatocellular tissue are essential for studying and understanding the regularities of homeostatic mechanism of tissue regulation of cholangiocyte proliferation during infestation of the organism by opisthorchias.

When studying the keilonic activity of an extract from liver tissue of inbred mice uninfested with opisthorchiasis, a significant decrease in proliferation in the experimental group was found by 60.1% (251.5±70.3 impulses/100 sec. in comparison with 630.9±93.3 impulses/100 sec. in the control, $p<0.05$). Kidneys were used as a test object for the tissue specificity of the keilon extract from liver tissue. The mitotic activity of cells of this organ in the control and experimental groups is statistically unreliable ($p>0.05$). Thus, the extract isolated from the tissue of the non-invasive liver fluke *Opisthorchis felineus* has pronounced tissue-specific keilon activity and can be used to perform experimental studies.

The results of the study of the sensitivity of hepatocytes in opisthorchiasis infestation lasting 2 months (♂ CBA/Lac) to the keilon factor isolated from healthy liver tissue (conducted intravenously at a dose of 10 mg per mouse) showed a difference with non-invasive animals. In mice with opisthorchiasis, DNA synthesis in hepatocytes is inhibited by keilon factor by 20.0% (249.8 impulses/min: - keilon factor in comparison with 200.4 impulses/min: + keilon factor), and in the control group (animals are not infested) the sensitivity of hepatocytes to keilon factor is slightly higher – the inhibition of proliferative activity was 27.8% (643.8 impulses/min: - keilon factor in comparison with 464.6 impulses/min: + keilon factor). The data obtained indicate that hepatocytes of non-helminth-infested animals are more sensitive to the action of a proliferation inhibitor isolated from healthy liver tissue than hepatocytes of animals infested by trematodes for 2 months (lower by 7.8%).

The biological activity of keilon factor isolated from the liver of mice at the above-mentioned period of opisthorchiasis infestation has a tendency similar to the sensitivity of hepatocytes to keilon factor. When exposed to a keilon-containing extract obtained from the liver of mice with 2-month opisthorchiasis infestation, there is a lower percentage of proliferation inhibition (609.8 impulses/min) – 66.8% than when exposed to keilon factor

isolated from the liver of non–invasive animals (387.5 impulses/min) - 78.9% relative to the control (1836.2 impulses/min). Based on the data obtained, it can be concluded that a 2-month opisthorchiasis infestation affects the activity of the keilon-containing liver extract. In almost healthy animals, keilon factor inhibits the proliferation of tissue cells more markedly than in infested animals (lower by 12.1%).

In practice, a decrease in the level of sensitivity of hepatocytes to the keilon factor in opisthorchiasis infestation (7.8%) and the biological activity of keilon factor isolated from the liver of mice infected with opisthorchiasis (by 12.1%) have the same tendency.

Thus, the results of the study showed that with prolonged opisthorchiasis infestation, activation of the proliferation of body tissues is associated with a violation of its regulation - a decrease in the activity of the membranotropic homeostatic factor (bioregulator) inhibiting proliferation (keilons, keilon factors) and the sensitivity of somatic cell receptors to it.

Deviation from the norm of the mechanisms of regulation of cellular and tissue homeostasis, induced by the infestation of the biotic pathogen Opisthorchis felineus, mediates a significant decrease in the functions of balanced control of the mitotic cycle and the process of cell differentiation. This is a factor determining the occurrence and development of the oncogenesis process [21].

The obtained results of experimental studies of the activity of the keilon-containing extract and the sensitivity of somatic cells to it in opisthorchiasis on the liver model of inbred mice suggest similar violations of the regulation of cell proliferation of other tissues, including the ductal epithelium. That is, a violation of the mechanism of biological action of antiproliferative signals of the keilon-containing factor through transmembrane somatic cells receptors leads to the proliferation of cells that are insensitive to growth-suppressing factors, which is a possible promoter (transmitter) of cholangiocancerogenesis.

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A change in the concentration or activity of keilon factor following the carcinogenic effect of any substances has been revealed by many authors, and at the stage of formation of tumor nodules this mechanism is disrupted [11, 21-22]. Probably, activation of cytomembrane lipid peroxidations and their destabilization plays a role in decreasing the sensitivity of somatic cell receptors to keilon factor [20].

The violation of the regulation of tissue homeostasis of the body during the infestation of the hepatobiliary system of mice by the trematode Opisthorchis felineus towards the activation of proliferation processes is confirmed by the following data. It has been shown that the growth rate of syngeneic malignant tumors in chronic opisthorchiasis relative to comparison group increases significantly: infestation 1 month, F1 [CBA/Lac x C57B1/6], malignant tumors cervical cancer-5 - by 33.3% ($p<0.05$); infestation 4.5 months, CBA/Lac, malignant tumor cervical cancer -5 - by 29.7% ($p<0.05$); infestation for 4 months, ♀ Balb/s,malignant tumor ACATOL - by 650.0% ($p<0.001$). The obtained results of the study are significant for assessing the role of a violation of the regulation of tissue homeostasis of the body in comparison with the background of opisthorchiasis infestation in determining the process of cholangiocancerogenesis.

In addition, it should be noted that under tissue stress, the interferon system, which is formed by genes, receptors, and effector molecules, is involved in the regulation of cell-tissue homeostasis (cell proliferation and differentiation processes) [23-25]. Among all types of interferons, leukocyte interferon (L- interferon/ interferon -α) is one of the most important for body homeostasis and pharmacology, accounting for 95.0% of all interferon molecules [26-27]. L- interferon circulates freely in the blood and spreads rapidly to organs and tissues. The interaction of L- interferon with tissue cells is realized through specific membrane receptors, enzymatic systems [9, 28], providing signal transmission to the cell nucleus for the realization of biological effects [29-30].

Among 300 effects of the interferon system, L-interferon has antiproliferative and antitumorigenic effects, in addition to antiviral, antimicrobial, immunomodulatory and others [27]. Currently, L-interferon based preparations are used in tumors of different histological structure and localization to prevent tumor progression, recurrences and metastases. The interferon system is considered to be an inhibitory regulator of almost all links of oncogenesis: initiation, proliferation, progression and metastasis [31-34]. The effect of inhibition of proliferation processes by this cytokine covers all cells of the organism, but the maximum sensitivity is observed in rapidly proliferating cells [35]. It is also known that L-interferon can inhibit certain oncogenes (c-myc, c-src, cHa-ras) in tumor cells and induce activation of other genome regions that can provide proapoptotic activity [36]. An essential effect of L-interferon is its ability to induce maturation processes in non-differentiated tumor cells while inhibiting their proliferation [35, 37].

Previously, we have shown [38] that intraperitoneal injection of transformed cell growth-inhibitory factor (L-interferon) at a dose of $5 \times 10^3$ units into inbred tumor-bearing ♀ CBA/Lac mice with subcutaneous (0.5 ml/1 x $10^6$ cells) inoculation of a syngeneic tumor of cervical cancer-5 infected with the biotic pathogen *Opisthorchis felineus* for 4.5 months resulted in an increase in the growth rate of the tumor by 104.0 % (2 g) and by 133.7 % (1 g) over 20 days compared to infested tumor-bearing mice (1 g) for 20 days, an increase in the growth rate of the indicated tumor by 104.0 % (2 g) was observed in comparison with infested tumor-bearing mice (1 g) and by 133.7 % exceeded this index in the group (3 g) of uninfected tumor-bearing animals.

It is necessary to pay attention to the fact that with intraperitoneal conducting of homologous leukocyte L-interferon at the indicated dose and period to inbred non-cholinergic mice, ♂ TWO/2 and ♀ CBA/Lac with a period of opisthorchiasis infestation of 4 months, the epithelium of the intrahepatic ducts significantly decreased by 20.0% and 55.5 %, respectively. That is, the studied growth-inhibiting cytokine L-interferon competes to a certain extent with the growth factors of opisthorchiasis.

Literature analyses shows that L-interferon inhibits the proliferative activity process in all tissues, including carcinomas [35, 37]. The nature of the mechanisms of stimulation of tumor tissue cell growth when L- interferon is introduced into the organism remains unclear. However, it is known that tumor cells have defects in the regulatory chain of

proliferation. In carcinogenesis there is hyperexpression of cell receptors possessing tyrosine kinase activity to growth factors, i.e. regulation of cell surface receptors is disturbed and transduction of mitogenic signals inside the cell increases significantly. Probably, significant self-sufficiency of malignant tumor with growth signals (mitogenic signals) in comparison with the background of high level of helminth growth factors circulating in the environment of the organism taking into account hyperexpression of membrane receptors tropic to them determines certain features of biological action of somatic and malignant cells proliferation inhibitor L-interferon. Probably, growth factors secreted by opisthorchias and tumor tissue synergistically compete with L-interferon, prevailing in biological action on tissues - activate proliferative processes in carcinomas. In the absence of malignant tumors in opisthorchis-infected mice, homologous L-interferon has a certain growth-inhibitory effect on somatic cells.

The obtained data indicate that in the presence of malignant tumor in the body in comparison with the background of long-term opistorchiasis infestation, the effect of malignant growth inhibitor - homologous leukocyte interferon (L- interferon) - is modified into a stimulant [12].

Modification of the antitumorigenic effect of L-interferon under the influence of metabolites of opisthorchiasis (growth factors) and carcinoma growth factors released into the body environment towards activation of the malignant process is possible only at the level of regulation of cellular genome function.

## 4 Conclusion

Prolonged parasitization in the hepatobiliary system of the organism by the ecopathogenic helminth Opisthorchis felineus mediates a disruption of the mechanisms of regulation of cell-tissue homeostasis towards a decrease in the biological function of the growth-inhibitory tissue-specific keilon-containing factor, i.e. a decrease in the control over the mitotic cycle of somatic cells. This causes high proliferative activity of cholangiocytes and other somatic cells of the organism.

Disturbance of mechanisms of regulation of cell-tissue homeostasis of the organism is confirmed by the phenomenon of modification of biological action of homologous leukocytic interferon in inbred tumor-bearing mice with chronic opisthorchiasis - from inhibitor of carcinogenesis to activator. The established pattern suggests the exclusion of L- interferon use in chronic opisthorchiasis patients with malignant processes.

Decrease in the activity of growth-inhibitory tissue-specific keilon-containing factor and sensitivity to it of somatic cells in comparison with the background of chronic infestation of the organism by the helminth *Opisthorchis felineus* when the tissues of ductal epithelium are exposed to mutagens (carcinogens) plays the role of a promoter of malignant tumors, including cholangiocarcinogenesis.

One of the tasks of secondary prevention of cholangiocarcinogenesis in the posthelminthic period is the development of biotechnological methods that restore the systems of regulation of cell-tissue homeostasis of the organism, i.e. control of the mitotic cycle and the process of somatic cell differentiation.

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