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IL-4 И ЕГО ПОЛИМОРФИЗМ (*IL4*-589C/T) ПРИ ЦЕРВИКАЛЬНОЙ НЕОПЛАЗИИ

Абакумова Т.В.[1], Мягдиева И.Р.[1], Долгова Д.Р.[1], Генинг С.О.[1], Антонеева И.И.[1, 2], Генинг Т.П.[1]

[1] ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия
[2] ГУЗ «Областной клинический онкологический диспансер», г. Ульяновск, Россия

Резюме. Переход неоплазии шейки матки (CIN) в рак шейки матки происходит при активном участии IL-4, для которого показано как про-, так и противоопухолевое действие при опухолях различной локализации. Экспрессия цитокинов регулируется на уровне транскрипции в промоторной области гена. Показано, что генотип *IL4* (589C/T) (rs2243250) ассоциирован с развитием рака желудка и молочной железы. Вклад вариаций генотипа *IL4* в развитие CIN еще не изучен. Цель исследования – оценить риск развития неоплазии шейки матки по наличию полиморфизма *IL4* (589C/T) и уровню IL-4.

Объектом исследования служили циркулирующие нейтрофилы, сыворотка и геномная ДНК 36 больных CIN и 20 женщин без дисплазии (группа сравнения). С помощью ИФА определяли уровень IL-4 в лизате нейтрофилов и сыворотке крови. Оценивали фагоцитарную активность и способность нейтрофилов к адгезии (CD11b). Аллель-специфическую ПЦР в реальном времени с использованием зондов Taq-Man использовали для анализа *IL4* 589C/T (rs2243250). Статистическую обработку проводили с помощью программ Statistica 13 и Jamovi 1.6.5.0.

В результате исследования установлено, что уровень IL-4 в сыворотке крови и циркулирующих нейтрофилах у больных с CIN достоверно выше, чем в группе сравнения. Аллель -589C* гена *IL4* и генотип TT чаще встречаются в группе с CIN (55,5%), чем в контроле (25%). При этом установлена прямая связь между наличием полиморфизма и повышенной адгезивной способностью и с показателями фагоцитарного числа циркулирующих нейтрофилов. Анализ частоты встречаемости *IL4* C589T методом «случай-контроль» показал, что шансы формирования CIN у носителей аллеля -589C и генотипа TT составили 3,75 (95% ДИ: 1,013-13,880, Хи-квадрат = 4,161, p = 0,042). Аллель -589C* и TT генотип *IL4*, уровни нейтрофилов и сывороточного IL-4 связаны с инфекцией ВПЧ. С помощью модели бинарной логистической регрессии показана возможность использования уровней IL-4 в циркулирующих нейтрофилах и полиморфизма *IL4* (589C/T) для дифференциальной диагностики пациентов с CIN ($\chi^2 = 15,6$, p = 0,001). Значимость их сочетания оценивали с помощью анализа ROC-кривых (IL-4 в нейтрофилах; *IL4* (-589C*), вероятность 75%).

Адрес для переписки:

Мягдиева Ильсея Ринатовна
ФГБОУ ВО «Ульяновский государственный университет»
4321017, Россия, г. Ульяновск, ул. Л. Толстого, 42.
Тел.: 8 (937) 039-85-86.
E-mail: ilseya2015@yandex.ru

Address for correspondence:

Ilseya R. Myagdieva
Ulyanovsk State University
42 Leo Tolstoy St
Ulyanovsk
432017 Russian Federation
Phone: +7 (937) 039-85-86.
E-mail: ilseya2015@yandex.ru

Образец цитирования:

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Таким образом, *IL-4* (589C/T) связан с адгезивной и фагоцитарной активностью циркулирующих нейтрофилов. У ВПЧ-инфицированных пациентов полиморфизм гена *IL-4* (589C/T) может служить маркером раннего выявления и прогноза CIN.

Ключевые слова: IL-4, полиморфизм IL4 (589C/T), цервикальная интраэпителиальная неоплазия, нейтрофилы, ВПЧ, дифференциальная диагностика

IL-4 AND ITS POLYMORPHISM (*IL4*-589C/T) IN CERVICAL NEOPLASIA

Abakumova T.V.[a], Myagdieva I.R.[a], Dolgova D.R.[a], Gening S.O.[a], Antoneeva I.I.[a, b], Gening T.P.[a]

[a] *Ulyanovsk State University, Ulyanovsk, Russian Federation*
[b] *Regional Clinical Oncologic Center, Ulyanovsk, Russian Federation*

Abstract. The transition of cervical neoplasia (CIN) to cervical cancer occurs with the active participation of IL-4, for which both pro- and antitumor effects have been shown with tumors of various localizations. The expression of cytokines is regulated at the transcriptional level in the promoter region of the gene. It has been shown that the genotype *IL4* (589C/T) (rs2243250) is associated with the development of gastric and breast cancer. The contribution of IL-4 genotypic variations to the development of CIN has not yet been studied. The aim of the study was to assess the risk of developing cervical neoplasia by the presence polymorphism of *IL4* (589C/T) and the level of IL-4. The object of the study was circulating neutrophils, serum and genomic DNA of 36 patients with CIN and 20 women without dysplasia (comparison group). Using ELISA, the level of IL-4 was determined in neutrophil lysate and serum. Phagocytic activity and adhesive ability (CD11b) of neutrophils were assessed. Allele-specific real-time PCR using Taq-Man probes was used to analyze of the *IL4* 589C/T (rs2243250). Statistical processing was carried out using Statistica 13 and Jamovi 1.6.5.0. As a result of the study, it was found that the level of IL-4 in serum and circulating neutrophils in patients with CIN is significantly higher than in the comparison group. The -589C* allele of the *IL4* gene and the TT genotype are more common in the group with CIN (55.5%) than in the control (25%). At the same time, a direct relationship was established between the presence of polymorphism and increased adhesive ability and with indicators of the phagocytic number of circulating neutrophils. Analysis of the incidence of *IL4* C589T by the «case-control» method showed that the chances of CIN formation in carriers of the -589C allele and the TT genotype were 3.75 (95% CI: 1.013 – 13.880, Chi-square = 4.161, p = 0.042). The -589C* allele and TT *IL4* genotype, neutrophil and serum IL-4 levels are associated with HPV infection. Using a binary logistic regression model, we demonstrated the possibility of using IL-4 levels in circulating neutrophils and IL-4 gene polymorphism (589C/T) for the differential diagnosis of patients with CIN ($\chi^2 = 15.6$, p = 0.001). Significant significance for their combination was assessed by ROC-curve analysis (IL-4 in neutrophils; *IL4* (-589C*), 75% probability. Thus, the *IL4* (589C/T) is associated with the adhesive and phagocytic activity of circulating neutrophils. In HPV-infected patients, *IL4* gene polymorphism (589C/T) can serve as a marker for early detection and prognosis of CIN.

Keywords: IL-4, IL4 polymorphism (589C/T), neutrophils, cervical intraepithelial neoplasia, HPV, differential diagnosis

Introduction

Infection with certain strains of human papillomavirus (HPV) is associated with a high risk of malignant transformation, and HPV-associated cervical intraepithelial neoplasia (CIN) can become an invasive cancer. Up to 32% of cases there is a transition from CINIII to cervical cancer [2]. In this case, factors that regulate tumor growth and modulate immunological control can play a special role. Interleukin 4 (IL-4) has an antitumor effect [13], however elevated levels of IL-4 have been found in tumor tissues in patients with breast, renal cell, prostate, colon and lung cancers [8, 11]. IL-4 is mainly produced by macrophages and T lymphocytes [1], as well as by neutrophils (Nph) [3]. At the same time, it causes hyperproduction of antibodies, which leads to

increased tumor growth due to "antibody screening" of tumor cell antigens [9]. At the same time, the tumor growth-inhibiting effect of IL-4 associated with blockade of the cell cycle, increased expression of MHC on tumor cells, and a decrease in the expression of oncogenes was shown [12].

The expression of cytokines is regulated at the transcriptional level in the promoter region of the gene. The functional polymorphism of the promoter region of the *IL4* gene at position -590C → T and the association of increased protein production with the *T allelic variant of the gene were shown [10]. In a detailed subgroup analysis by cancer type, the *IL4* genotype (589C/T) (rs2243250) was associated with the risk of developing gastric and breast cancer [4, 14]. At the same time, Wang et al. (2012) showed that carriers of the Q576R G *IL4R* allele were associated with a significantly reduced risk of cervical cancer. To date, the contribution of *IL4* genotypic variations to the development of CIN has not been studied.

The aim of the study was to assess the risk of developing cervical neoplasia by the presence of *IL4* polymorphism (589C/T) and the level of IL-4 in circulating neutrophils.

Materials and methods

The study included 36 patients with verified CIN who were examined and then treated at the Regional Clinical Oncological Center in Ulyanovsk, and 20 women without dysplasia (comparison group), corresponding in age to patients in the study group and not having a history of oncological diseases (Table 1). The study was conducted in compliance with the principles of voluntariness and confidentiality, in accordance with the requirements of the ethics commission of the Institute of Medicine, Ecology and Physical Culture of Ulyanovsk State University (protocol No. 3, dated March 15, 2015).

The level of IL-4 was determined by ELISA in the Nph lysate and serum before the start of treatment (CJSC Vector-Best-Volga, Russia). Genomic DNA was isolated from peripheral blood leukocytes using the DNA Express Blood kit (Litekh, Russia). The study of the phagocytic activity of Nph was carried out by quantitative determination of the absorption and digestion capacity of Nph during 30 min incubation with *Saccharomyces cerevisiae*. The ability of Nph to adhere was assessed by the expression of the surface marker CD11b. Allele-specific real-time PCR using Taq-Man probes was used to analyze the polymorphic variant of the promoter region of the *IL4* gene 589C/T (rs2243250) (Litekh, Russia).

The frequencies of alleles and genotypes of polymorphic loci, as well as the correspondence of the distribution of the observed frequencies of genotypes to those theoretically expected according to the Hardy–Weinberg equilibrium, were checked using the χ^2 test. To assess the relative risk of developing a disease/event, the OR value (odds ratio) was calculated in case-control studies. Sets of quantitative indicators, the distribution of which differed from normal, were described using the values of the median (Me) and the lower and upper quartiles ($Q_{0.25}$-$Q_{0.75}$). The Mann–Whitney U test was used to compare independent populations in cases where there were no signs of normal data distribution. The construction of a predictive risk model for the outcome of a malignant neoplasm was performed using the binary logistic regression method. The statistical significance of the resulting model was determined using the χ^2 test. The quality of the predictive model obtained using ROC

TABLE 1. CHARACTERISTICS OF PATIENTS INCLUDED IN THE STUDY

Characteristic	Number of patients (%)
Mean age, median 51.5 years (IQR, $Q_{0.25}$-$Q_{0.75}$; 39-59)	n = 56 (100%)
Diagnosis :	
Without dysplasia	20 (35,7%)
CIN (dysplasia II-III, cancer in situ)	36 (64.3%)
HPV-positive	52 (92,8%)
Complete blood count of patients with CIN before treatment: leukocytes, *10^9/L, median 6.2 (IQR, $Q_{0.25}$-$Q_{0.75}$; 5.2-7.7) neutrophils, *10^9/L, median 10.6 (IQR, $Q_{0.25}$-$Q_{0.75}$; 8.6-11.6)	

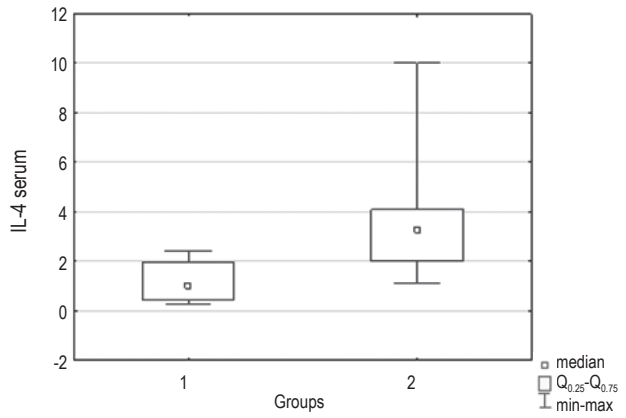

Figure 1. Level of IL-4 in the blood serum of the comparison group (1) and patients with CIN (2)

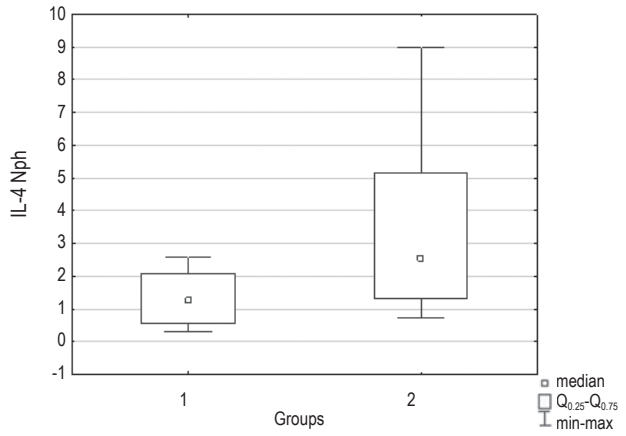

Figure 2. IL-4 level in circulating neutrophils of the comparison group (1) and patients with CIN (2)

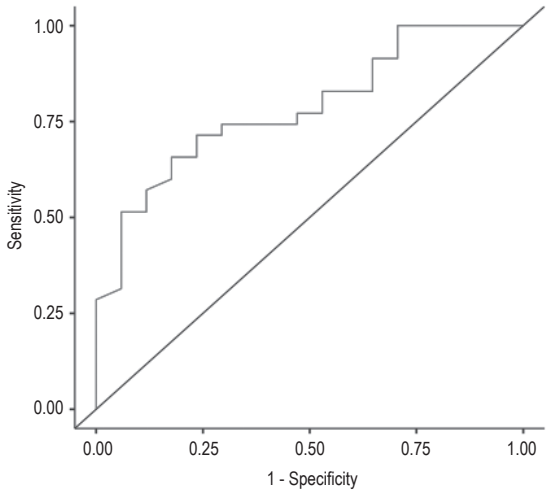

Figure 3. ROC-curve for the regression model of the differential diagnosis of CIN, taking into account the indicators: the level of IL-4 in neutrophils and *IL4* (-589C*)

analysis was assessed based on the area under the ROC curve with a standard error and 95% confidence interval (CI) and the level of statistical significance (Jamovi 1.6.5.0). Statistical processing was carried out using Statistica 13.

Results and discussion

Today, there is an opinion that IL-4 is a "tumor-promoting molecule". Serum levels of IL-4 are usually elevated in cancer patients [7, 15]. According to Harris et al. (2019), through IL-4 receptors have a direct stimulating effect on epithelial tumor cells. As a result of the study, we also found a significantly increased serum level of IL-4 ($p = 0.003$) in patients with CIN compared with the values in the comparison group (Figure 1).

The relationship between IL-4 and circulating Nph seems to be quite complex and poorly understood. An analysis of literature data suggests that IL-4 actively influences the morphofunctional state of Nph: it limits the migration of Nph into tissues, inhibits its survival in tissues, delays their apoptosis, activates *de novo* actin synthesis and rearrangement of the Nph cytoskeleton [5]. According to Guo et al. (2013), IL-4 also recruits Nph through the activation of cytokine-induced chemoattractant and adhesion molecules. We found a significant increase in the level of IL-4 in circulating Nph ($p = 0.0008$) compared with the control group (Figure 2).

A study of the polymorphism of the promoter gene, which determines the level of IL-4 production in patients with neoplastic processes, showed an association of IL-4 SNP with the risk of developing bladder cancer, gastric and pancreatic cancer, and metastatic kidney cancer [6]. At the same time, both an increase and a decrease in the frequency of the TT genotype in patients with breast cancer were noted [10]. Analysis of the polymorphism of the *IL4* promoter region (589C/T) in patients with CIN has not been performed to date. In our study, the -589C* allele of the *IL4* gene and the TT genotype were more common in the group with CIN (55.5%) than in the control (25%).

At the same time, a direct relationship was established between the presence of polymorphism and an increase in adhesive ability ($r = 0.409$, $p = 0.004$) and with indicators of the phagocytic number of circulating Nph ($r = 0.428$, $p = 0.002$). Analysis of the incidence of functional polymorphism C-589T of the *IL4* gene by the "case-control" method showed that the chances of CIN formation in carriers of the -589C allele and the TT genotype were

3.75 (95% CI: 1.013 – 13.880, Chi-square = 4.161, p = 0.042). Allele -589C* and TT *IL4* genotype (r = -0.397, p = 0.004), IL-4 level in Nph (r = 0.386, p = 0.009) and in serum (r = 0.458, p = 0.0002) are associated with HPV infection. The level of IL-4 in Nph and serum is not associated with the C-589T polymorphism of the *IL4* gene.

Using a binary logistic regression model, we demonstrated the possibility of using indicators of the level of IL-4 in circulating Nph and *IL4* gene polymorphism (589C/T) for the differential diagnosis of patients with CIN ($\chi^2 = 15.6$, p = 0.001). Significant significance in their combination was assessed by ROC-curve analysis (IL-4 in Nph, OR 1.693 95% CI 1.048-2.74, p = 0.032; *IL4* (-589C*), OR 4.317 95% CI 1.059-17.60, p = 0.041). The area under the curve (AUC) of this model was 0.785, and CIN could be diagnosed with a 75% probability (Figure 3). The values of the selected indicators allow us to classify patients according to the degree of risk of CIN in combination with sensitivity (0.800) and specificity (0.471).

Conclusion

The *IL4* gene polymorphism (589C/T) is associated with the adhesive and phagocytic activity of circulating Nph. In HPV-infected patients, *IL4* gene polymorphism (589C/T) can serve as a marker for early detection and prognosis of CIN.

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Авторы:

Абакумова Т.В. *— д.б.н., доцент, профессор кафедры физиологии и патофизиологии Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Мягдиева И.Р. *— ассистент, аспирант кафедры физиологии и патофизиологии Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Долгова Д.Р. *— к.б.н., доцент, доцент кафедры физиологии и патофизиологии Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Генинг С.О. *— к.м.н., старший преподаватель кафедры физиологии и патофизиологии Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Антонеева И.И. *— д.м.н., профессор, профессор кафедры онкологии и лучевой диагностики ФГБОУ ВО «Ульяновский государственный университет»; заведующая гинекологическим отделением ГУЗ «Областной клинический онкологический диспансер», г. Ульяновск, Россия*

Генинг Т.П. *— д.б.н., профессор, заведующая кафедрой физиологии и патофизиологии ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Authors:

Abakumova T.V., *PhD, MD (Biology), Associate Professor, Professor, Department of Physiology and Pathophysiology, Institute of Medicine, Ecology and Physical Culture, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Myagdieva I.R., *Assistant Professor, Postgraduate Student, Department of Physiology and Pathophysiology, Institute of Medicine, Ecology and Physical Culture, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Dolgova D.R., *PhD (Biology), Associate Professor, Department of Physiology and Pathophysiology, Institute of Medicine, Ecology and Physical Culture, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Gening S.O., *PhD (Medicine), Assistant Professor, Department of Physiology and Pathophysiology, Institute of Medicine, Ecology and Physical Culture, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Antoneeva I.I., *PhD, MD (Medicine), Professor, Department of Oncology and Radiation Diagnostics, Ulyanovsk State University; Head of the Gynecological Department, Regional Clinical Oncologic Center, Ulyanovsk, Russian Federation*

Gening T.P., *PhD, MD (Biology), Professor, Head, Department of Physiology and Pathophysiology, Ulyanovsk State University, Ulyanovsk, Russian Federation*

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