ДЕПРИВАЦИЯ VEGF ВЛИЯЕТ НА ЭКСПРЕССИЮ ЭНДОГЛИНА В КЛЕТКАХ ТРОФОБЛАСТА И ЕСТЕСТВЕННЫХ КИЛЛЕРАХ

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Резюме. Белки семейства VEGF участвуют в развитии многих клеточных популяций: эндотелиальных клеток, моноцитов и макрофагов, стволовых клеток, опухолевых клеток, мышечных клеток стенок сосудов, клеток трофобласта и в целом любых клеток, экспрессирующих рецепторы к VEGF. Нарушения, затрагивающие продукцию белков VEGF и проведение сигналов от них, приводят ко многим патологическим состояниям, в том числе к аномалиям развития плаценты. Клетки трофобласта являются основной популяцией клеток, формирующей плаценту. Они вовлечены в процессы секреции и рецепции VEGF, фактора, необходимого для обеспечения ангиогенеза. Несмотря на это, на данный момент в литературе недостаточно данных о влиянии проведения сигналов от VEGF в клетках трофобласта на их функциональные особенности. Среди клеток окружения трофобласта, которые могут воздействовать на их активность в ходе беременности, особой группой являются материнские иммунные клетки, в частности NK-клетки. Принимая во внимание высокую численность NK-клеток в децидуальной оболочке, необходимо учитывать их вклад в изменение фенотипа клеток трофобласта. В настоящем исследовании изучалась экспрессия NK-клетками и клетками трофобласта белков MICA и MICB, а также рецептора CD105. Молекулы MICA и MICB являются маркерами стресса и позволяют судить о жизнеспособности клеток. Рецептор CD105 экспрессирован на поверхности некоторых популяций клеток и участвует в передаче сигнала от белков семейства TGF-β. В частности, показано, что эндоглин регулирует сигналинг от TGF-β путем направления сигнала через пути SMAD2/3 или SMAD1/5/8. Эндоглин, согласно литературе, ингибирует сигналинг, задействующий белок SMAD3. Играет ли эндоглин ту же роль в случае NK-клеток и трофобласта, неизвестно. Изучение изменений в экспрессии эндоглина является актуальной проблемой, поскольку сигналы от TGF-β необходимы при дифференцировке популяций трофобласта, а нарушения в механизмах сигналинга могут приводить к невынашиванию. В результате исследования мы показали, что VEGF играет роль в регуляции активности трофобласта и естественных киллеров. В частности, депривация

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Образец цитирования:

Е.В. Тыщук, Е.А. Денисова, О.Б. Марко, И.Ю. Коган, С.А. Сельков, Д.И. Соколов «Депривация VEGF влияет на экспрессию эндоглина в клетках трофобласта и естественных киллерах» // Медицинская иммунология, 2025. Т. 27, № 5. С. 1099-1110. doi: 10.15789/1563-0625-VDA-2991

For citation:

E.V. Tyshchuk, E.A. Denisova, O.B. Marko, I.Yu. Kogan, S.A. Selkov, D.I. Sokolov "VEGF deprivation affects endoglin expression in trophoblast cells and natural killers", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2025, Vol. 27, no. 5, pp. 1099-1110. doi: 10.15789/1563-0625-VDA-2991

DOI: 10.15789/1563-0625-VDA-2991

VEGF-A моноклональными антителами против этого цитокина при сокультивировании трофобласта и NK-клеток приводит к угнетению экспрессии CD105 обеими популяциями клеток. При этом суточная инкубация трофобласта с антителами к VEGF не вызывала изменений в их устойчивости к цитотоксической активности естественных киллеров. Вместе полученные результаты говорят о том, что депривация VEGF приводит к значимым изменениям в рецепции белков семейства TGF-β клетками трофобласта и естественными киллерами.

Ключевые слова: antiVEGF, CD105, эндоглин, NK-клетки, трофобласт, TGF-β

VEGF DEPRIVATION AFFECTS ENDOGLIN EXPRESSION IN TROPHOBLAST CELLS AND NATURAL KILLERS

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Abstract. Vascular Endothelial Growth Factors (VEGFs) are a group of proteins involved in differentiation of various cell types including endothelial cells, monocytes, macrophages, stem cells, tumor cells, vascular smooth muscle cells, trophoblast cells, and other cell populations that express VEGF receptors. Pathological conditions, such as abnormalities in placental development, can be caused by altered production and signaling of VEGFs. Trophoblast cells play a significant role in placental formation and are essential for angiogenesis due to their secretion and reception of VEGF. However, there is a lack of information in the literature regarding the influence of VEGF on functional characteristics of trophoblast cells. Maternal immune cells, particularly natural killer (NK) cells, have been shown to affect the activity of trophoblasts during pregnancy. Given the high abundance of NK cells in decidual tissue, it is important to evaluate their potential influence on phenotype of trophoblast cells. In this study, we investigated the expression of MICA, MICB, and CD105 proteins by NK cells and trophoblast cells. MICA and MICB are stress markers that allow us to assess cell viability. CD105 is a receptor expressed on the surface of various cell types. It plays a role in signal transmission from TGF-β family proteins. In particular, endoglin has been shown to regulate signaling from TGF-β by directing signals through the SMAD2/3 or SMAD1/5/8 pathways. According to the literature, endoglin inhibits the SMAD3-mediated signaling. However, similar effects of endoglin have not been confirmed for NK cells and trophoblasts. The studies of endoglin expression levels are of importance, since the signals from TGF-β are essential for differentiation of trophoblast cells. Disruption of TGF-β signaling can lead to pregnancy complications and miscarriage. We have demonstrated that VEGF plays a role in regulating the activity of trophoblasts and NK cells. In particular, treatment with neutralizing monoclonal antibodies to VEGF-A was associated with reduced expression of CD105, a VEGF coreceptor, on trophoblasts and NK cells under co-culture conditions. However, pretreatment of trophoblasts with anti-VEGF antibodies did not alter their resistance to the cytotoxic activity of NK cells. Taken together, these findings suggest that inhibition of VEGF signaling results in significant changes in reception of TGF-β family proteins by trophoblasts and natural killer cells.

Keywords: antiVEGF, CD105, endoglin, NK cells, trophoblasts, TGF-β

This research was supported by the Fundamental Scientific Research project No. 122041500062-5, "Optimization of methods for predicting, preventing, and treating 'large obstetric syndromes' and delivery strategies for pregnant women in high-risk groups to improve obstetrical and perinatal outcomes", conducted at the D. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russian Federation

Introduction

Members of the VEGF family of proteins regulate vascular growth [3]. Many pathological conditions, including abnormalities of placenta development, are

associated with complications in the production of VEGF proteins and the transmission of signals from them [22]. There are three types of VEGF receptors: VEGFR1, which binds VEGF-A, VEGF-B, PlGF, and VEGF-F; VEGFR2, which binds VEGF-A, VEGF-E, VEGF-C, and VEGF-D; and VEGFR3, which binds VEGF-C and VEGF-D [28]. These receptors cause the activation of different signaling pathways in cells. The activation of VEGFR1 and VEGFR2 receptors is involved in the process of angiogenesis, both physiological and pathological, while VEGFR3 regulates the process of lymphangiogenesis [39].

Speaking about the participation of VEGFR1 and VEGFR2 in the regulation of angiogenesis, it should be noted that VEGFR2 plays a primary role in the activation of many processes in cells related to proliferation, migration and blood vessel formation. However, the involvement of VEGFR1 in these processes cannot be denied either, since experiments with the deletion of the murine gene encoding VEGFR1 resulted in pathological vascular development and the embryonic death [5, 11]. It has been shown that VEGFR1 has a much higher affinity for VEGF-A compared to VEGFR2. However, the level of phosphorylation of VEGFR1 after activation is lower than that of VEGFR2, which could explain the more active participation of VEGFR2 in the regulation of angiogenesis [17, 44]. Another characteristic of VEGFR1 is the existence of a soluble form of the receptor, sFlt1, which has an affinity for A-VEGF that is comparable to that of the membrane form [20]. Since increased VEGFR1 expression leads to a decrease in the concentration of VEGF-A available to bind to VEGFR2, VEGFR1 is thought to act as a regulator of signal transduction through VEGFR2 [26].

VEGF is involved in the development of various cell types, including endothelial cells, monocytes, macrophages, stem cells, tumor cells, vascular smooth muscle cells, trophoblast cells, and any other cells that express VEGF receptors [10, 28, 36]. VEGFR1 is also expressed by NK cells [6]. Many studies have shown high levels of VEGF-A expression in the placenta, particularly in macrophages, endometrial glandular cells, leukocytes, endothelial cells, vascular smooth muscle cells, in villous and extravillous trophoblasts, and in NK cells [8, 18, 19, 36, 37]. Disruption of VEGF signaling has been shown to be associated with pregnancy complications. For example, an increase in the concentration of sFlt1 in serum is a sign of preeclampsia, as it competes for VEGF binding with membrane VEGF receptors [7, 26, 51]. In addition, the intensity of VEGFR1 expression by syncytiotrophoblasts was found to be increased in cases of preeclampsia, compared to healthy pregnancies [51].

Maternal immune cells, particularly NK cells, are an important group of cells in the trophoblast microenvironment. During the first trimester, this cell population accounts for approximately 70% of all leukocytes within the decidua, highlighting the significance of their functions [1]. NK cells play a crucial role in the transformation of uterine spiral arteries and the regulation of trophoblastic invasion into the uterine mucosa [33, 48]. As already mentioned, both trophoblasts and NK cells have receptors for VEGF and are capable of secreting VEGF. Therefore, they are able to regulate each other's activity in an autocrine and paracrine way.

The interaction between NK cells and trophoblasts is one of the key factors supporting the pregnancy, as these cells are involved in the development of placental tissue. In this regard, the investigation of interactions mediated by both secretory products and receptors of trophoblasts and NK cells is of great importance. In particular, in the field of reproductive medicine, a lot of studies have been focused on the synthesis and reception of VEGF, a factor necessary for angiogenesis. However, despite this, there are currently insufficient data in the literature on the effect of VEGF on the functional characteristics of these cells [38]. Therefore, **the aim of this study** was to evaluate the role of VEGF in maintaining the viability of trophoblast and NK cells. To achieve this goal, bevacizumab was used. It is an antibody that binds to the VEGF-A and prevents it from binding to its receptors [32]. Bevacizumab is already widely used to treat choroidal neovascularization and diabetes complications [30], as well as various tumors and other abnormal angiogenesis-related conditions [12, 16, 34]. In this study, we investigated the surface markers of NK cells and trophoblasts, specifically the MICA and MICB proteins. These proteins function as stress markers and help determine cell viability. Additionally, we studied the CD105 receptor, which is expressed on endothelial cells, trophoblasts, and other cell types. This receptor plays a role in the transmission of signals from TGF-β family proteins [23].

It has been demonstrated that signals from TGF-β play a crucial role during placentation as they are essential for the differentiation of trophoblast populations [15, 49]. Disruptions in signaling pathways can lead to pregnancy loss [45]. Using an endothelial cell model, it has been shown that endoglin regulates TGF-β signaling by directing the signal via the SMAD2/3 or SMAD1/5/8 pathways. Signaling along the SMAD1/5/8 pathway promotes proliferation and migration of endothelial cells, thereby stimulating angiogenesis. In contrast, activation of the SMAD2/3

pathway has an anti-angiogenic effect [23]. Endoglin, according to the literature, inhibits the signaling pathway involving the SMAD3 protein [14]. Whether endoglin plays a similar role in NK cells and trophoblasts is currently unknown. However, it has been demonstrated that SMAD proteins, which are involved in these signaling pathways, are active in trophoblast cells [4, 47], and only SMAD2/3 have been identified in NK cells [50].

Materials and methods

Cell lines

The study was conducted using the JEG-3 and NK-92 cell lines (ATCC, USA), which reflect the main characteristics of extravillous trophoblasts and natural killer cells, respectively [13, 21]. The cells were cultured according to the manufacturer's instructions (ATCC, USA). Recombinant IL-2 ('Roncoleukin', BIOTECH, St. Petersburg, Russia) was used as a growth factor for the NK-92 cells. Cell viability in all experimental settings was assessed using trypan blue staining. The viability was at least 95% for each experiment.

Inductors

Antibodies to VEGF (5000 nM, Avastin, F. Hoffmann – La Roche Ltd., Switzerland, Germany) were used as inducers.

Assessment of the phenotype of JEG-3 and NK-92 cells after incubation in the presence of anti-VEGF antibodies

JEG-3 cells were cultured in 5 mL of medium in 25 cm flasks (BD Biosciences, USA) with a density of 1×10^6 cells, for 48 hours. After this period, 1.5×10^6 NK-92 cells were pre-treated with carboxyfluorescein diacetate succinimidyl ester (CFSE), in accordance with the manufacturer's instructions (Sigma-Aldrich, USA). The cells were then added to part of the flasks. NK-92 cells, stained with CFSE, and intact JEG-3 cells were used as controls. After that, the mono- and co-cultured cells were treated with antibodies to VEGF. After a 22-hour incubation period, the JEG-3 cells were removed from the flasks using a scraper without using a trypsin-versene solution. The cells were treated with Fc-block reagent (Miltenyi Biotec, Spain) and monoclonal antibodies against CD94, CD45, CD105, MICA, MICB, NKG2D, and NKG2A (R&D, BD Biosciences, USA) in accordance with the manufacturer's instructions. Appropriate isotypic antibodies (R&D, BD Biosciences, USA) were used as a control for non-specific binding. The expression of the markers and cell fluorescence intensity were evaluated using a FacsCantoII flow cytometer (BD Biosciences, USA). There were four biological replicates with one technical replicate for each experiment.

Assessment of the cytotoxic activity of NK-92 cells toward JEG-3 cells

The cytotoxic activity was assessed as described previously [29]. JEG-3 cells were cultured in a flask at a concentration of 2.5×10^5/10 mL of medium. After 2 hours, antibodies to VEGF were added to the flask. After culturing for 22 hours, the JEG-3 cells were washed and stained with a CASE solution following the manufacturer's instructions (Sigma-Aldrich, USA). The stained JEG-3 cells were removed from the flasks using trypsin and versene solution and then transferred to the wells of a 96-well round-bottom plate (BD Biosciences, USA). Next, NK-92 cells were added to the wells containing JEG-3 cells at a 10:1 ratio (effector:target). The plate was then centrifuged for 5 minutes at 100 g. After 4 hours of incubation, the cells were stained with a propidium iodide solution according to the manufacturer's instructions (Sigma-Aldrich, USA). The percentage of dead JEG-3 cells was assessed using a FacsCantoII flow cytometer (BD Biosciences, USA) following a previously described gating strategy [29]. There were three biological replicates and two technical replicates in each experiment.

Statistical analysis

GraphPad Prism 8 software was used for statistical analysis. Statistical comparisons between groups were conducted using a non-parametric Mann–Whitney U test. Differences were considered significant at $p < 0.05$.

Results

The expression of MICA and CD105 proteins by JEG-3 cells was altered in the presence of antibodies to VEGF and NK-92 cells

Analysis of the phenotype of JEG-3 cells revealed that approximately 12% of the cells express the MICB molecule, approximately 16% express MICA, and 18.5% express the CD105 receptor (Figure 1A).

The co-culture of JEG-3 cells with NK-92 cells, compared to monoculture, resulted in a two-fold increase in the percentage of JEG-3 cells expressing the CD105 receptor. The percentage of JEG-3 cells expressing MICA and MICB molecules remained unchanged under these conditions. Analysis of the mean fluorescence intensity after co-culture with NK-92 cells compared to monoculture showed no change in the expression intensity of MICA, MICB, and CD105 proteins by JEG-3. Analysis of the JEG-3 cells phenotype after its co-culture with NK-92 cells in the presence of antibodies to VEGF showed a decrease in the percentage of JEG-3 cells expressing MICA and CD105 proteins compared to the baseline level during co-cultivation, The percentage of JEG-3 cells

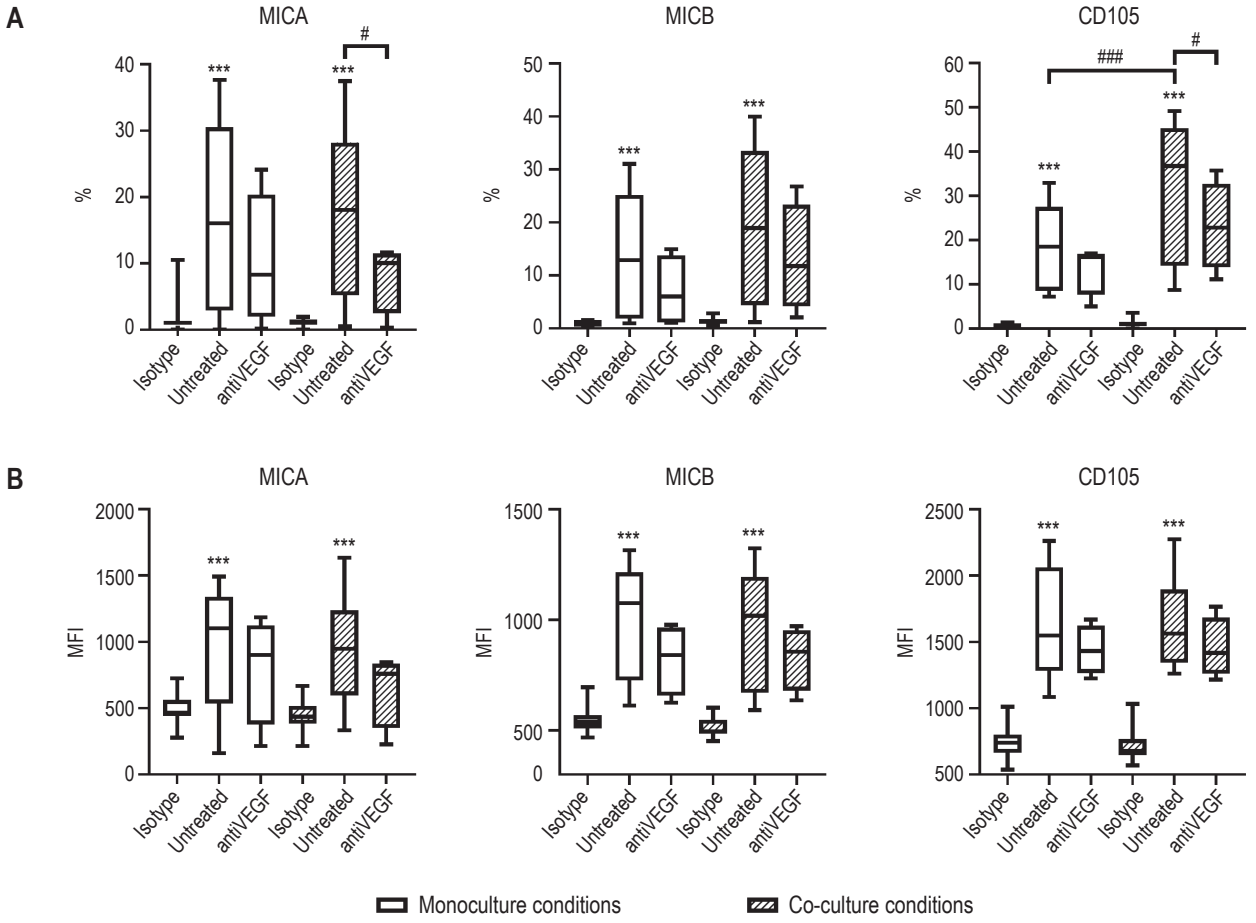

Figure 1. The percentage of trophoblast cells (JEG-3) expressing MICA, MICB, and CD105 proteins (A), and the intensity of expression of these markers (B) in the presence of antibodies against VEGF (antiVEGF) in mono- and co-culture with natural killer cells (NK-92)

Note. Differences from isotype control: ***, p < 0.001. Differences between groups: #, p < 0.05; ###, p < 0.001.

expressing the MICB receptor remained unchanged (Figure 1A).

Co-culture of JEG-3 cells with NK-92 cells, as well as treatment with antibodies to VEGF, did not affect the intensity of expression of MICA, MICB, and CD105 proteins by the cells (Figure 1B).

The phenotype of NK-92 cells was affected by the presence of antibodies to VEGF and JEG-3 cells

Analysis of the NK-92 cell phenotype has revealed that the entire population of studied cells expresses the NKG2D receptor on their surface and approximately 75% express the CD94 receptor. Additionally, the MICA protein has been found to be expressed on 1.5% of the cells, the MICB protein on 10.5%, and the CD105 protein on 26% (Figure 2).

When co-cultured with JEG-3 cells, NK cells reduced the expression level of CD94, compared to the level observed during monoculture, including in the presence of antibodies to VEGF. On the contrary, the percentage of NK-92 cells expressing the MICA protein increased under conditions of co-culture compared with monoculture. The percentage of NK-92 cells expressing NKG2D, MICB, NKG2A, and CD105 molecules under co-culture conditions did not change compared to monoculture (Figure 2). The cultivation of NK-92 cells in the presence of antibodies to VEGF led to a decrease in the number of cells expressing the CD105 receptor (Figure 2).

Co-culture of NK-92 cells with JEG-3 cells showed an increase in the intensity of expression of NKG2D, CD94, and CD105 receptors by NK-92 cells compared to monoculture. The results indicate functional activation of NK cells in the presence of target cells. Additionally, the intensity of expression of MICA and MICB proteins by NK cells also increased under co-culture conditions (Figure 3). Treatment of NK cells with antibodies to VEGF, both in mono- and coculture, leads to a decrease in the intensity of expression of the CD105 receptor (Figure 3). This

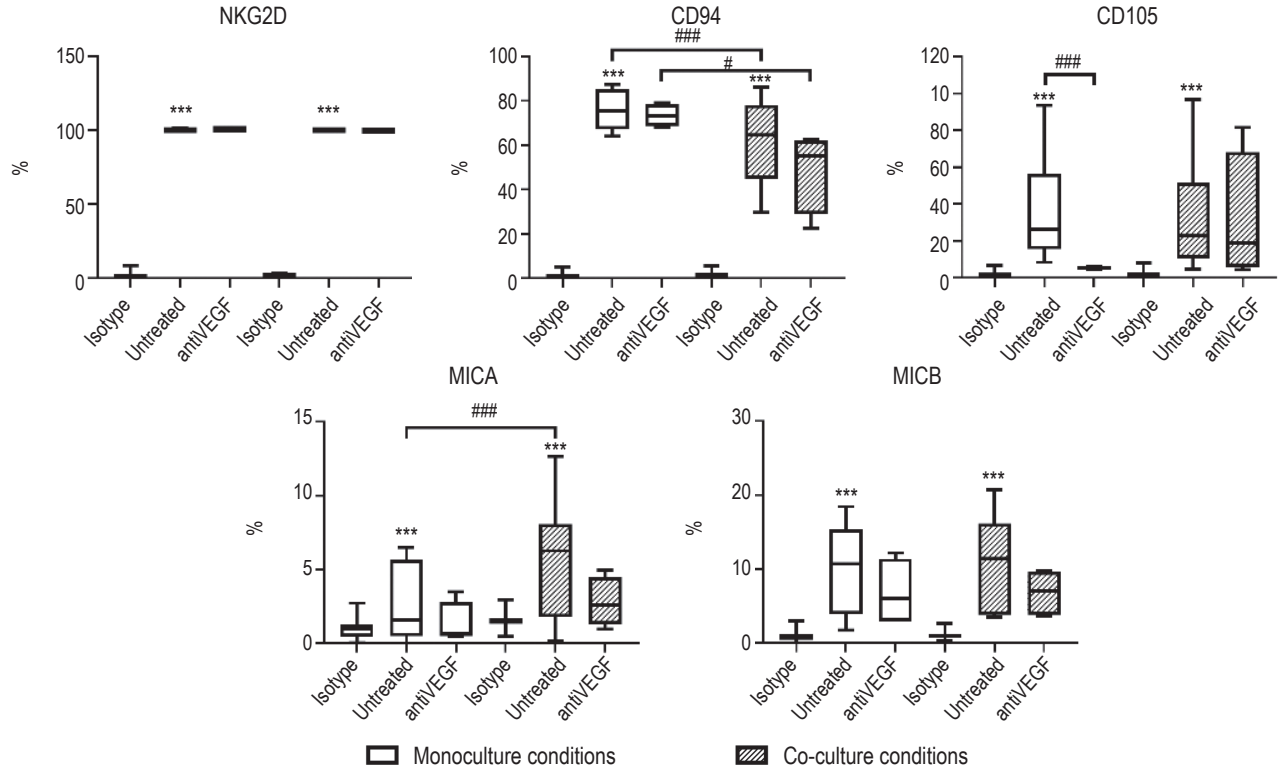

Figure 2. The percentage of natural killer cells (NK-92) expressing NKG2D, CD94, CD105, MICA, and MICB proteins in the presence of antibodies to VEGF (antiVEGF) in mono- and co-culture with trophoblast cells (JEG-3)

Note. Differences from isotype control: ***, p < 0.001. Differences between groups: #, p < 0,05; ###, p < 0,001.

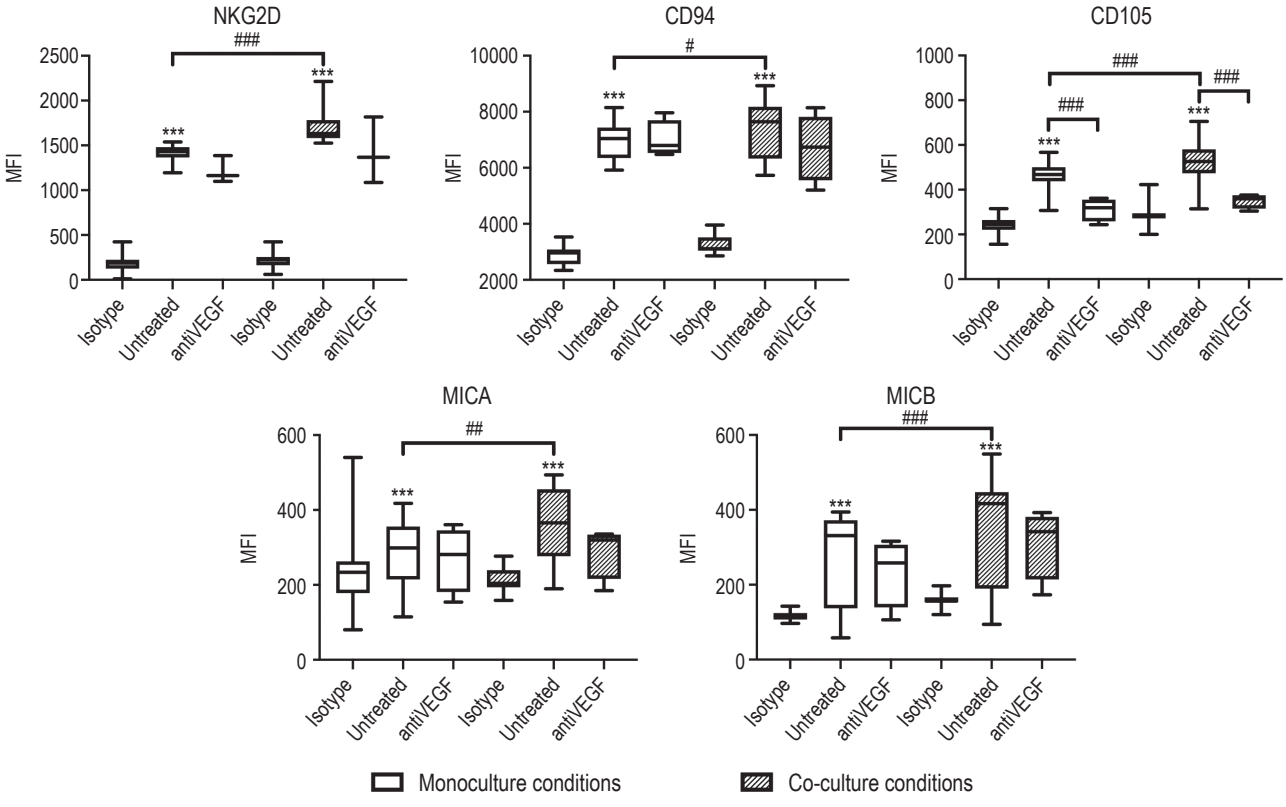

Figure 3. The intensity of expression of NKG2D, CD94, CD105, MICA, and MICB proteins by natural killer cells (NK-92) in the presence of antibodies against VEGF (antiVEGF) in mono- and co-culture with trophoblast cells (JEG-3)

Note. Differences from isotype control: ***, p < 0.001. Differences between groups: #, p < 0.05; ##, p < 0.01; ###, p < 0.001.

result may suggest changes in TGF-β signaling in the absence of VEGF.

Pretreatment of JEG cells with antibodies to VEGF did not affect their survival in the presence of NK cells

Analysis of the cytotoxic activity of NK-92 cells toward JEG-3 cells showed that, in the presence of NK cells, the mortality rate of JEG-3 was higher than the baseline mortality rate. Pretreatment of JEG cells with antibodies to VEGF did not influence their viability in the presence of NK cells (Figure 4).

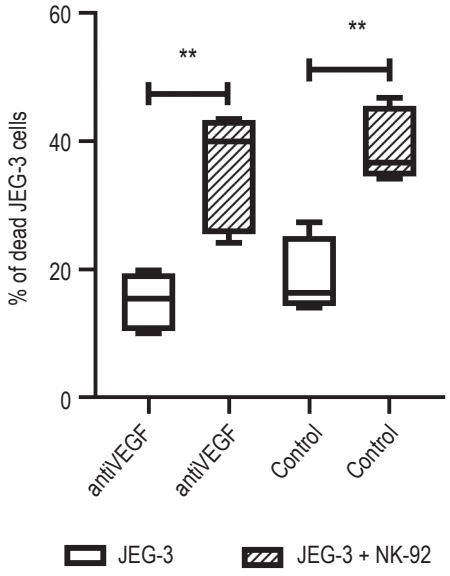

Figure 4. Cytotoxicity of NK-92 cells toward JEG-3 cells pretreated with antibodies to VEGF (antiVEGF)
Note. Significant differences: **, p < 0,01.

Discussion

Trophoblast cells and natural killer cells are important participants in the process of placentation. Both cell populations are capable of secretion and reception of VEGF. Previously, it has been found that VEGF affects the proliferation and survival of trophoblast cells [51]. VEGF also induces activation of NK cells adhesion [27]. Nevertheless, there is a lack of data in the literature on the role this factor plays in the cell activity. In this regard, we evaluated changes in the phenotype of JEG-3 trophoblast and NK-92 natural killer cells after their mono- or co-culture in the presence of antibodies to VEGF.

In this study, the expression of CD105, MICA, and MICB proteins was evaluated. Endoglin (CD105) is a coreceptor for TGF-β which regulates signal transmission from this factor via the SMAD2/3 or SMAD1/5/8 pathways [23, 24]. In particular, endoglin has been shown to activate signaling involving SMAD1/5/8 proteins and inhibit SMAD2/3 pathway [14, 35]. In endothelial cells, it has been demonstrated that signaling through the SMAD1/5/8 pathway promotes proliferation and migration of the cells, whereas activation of the SMAD2/3 pathway has an angiostatic effect [23]. Therefore, a high level of endoglin expression may indicate a more active transmission of signals through SMAD1/5/8 proteins compared to SMAD2/3. MICA and MICB are stress-induced molecules that are expressed by various cell populations, including immune cells [41]. An increase in the expression of these proteins was observed in tumor cells. MICA/B transcripts have been found in placental samples. The levels of mic mRNA are higher in samples taken from patients with preeclampsia compared to those from healthy patients [2, 42]. The analysis of the levels of expression of these markers allows us to assess the physiological state of trophoblast cells and monitor the conditions under which they experience stress.

In this work, we showed that JEG-3 trophoblast cells express all three markers. Under conditions of co-culture with NK cells, the number of trophoblast cells expressing endoglin increased. This result suggests a possible increased role for endoglin in mediating the transmission of TGF-β signals in trophoblast cells in the presence of natural killer cells. It also suggests a potential alteration in signaling pathways, from the SMAD2/3 pathway to the SMAD1/5/8, which may activate trophoblast proliferation. The treatment of the cell co-culture with antibodies to VEGF led to a decrease in the level of endoglin expression by trophoblasts to the initial level observed during monoculture. At the same time, under monoculture conditions, the endoglin level remained constant regardless of the presence of antibodies to VEGF. Apparently, TGF-β signaling is regulated by VEGF and its absence in the cell co-culture prevents the changes that occur in trophoblast cells when they are exposed to NK cells. It has also been shown that activation of SMAD2/3 in trophoblast cells leads to the secretion of VEGF-A [25]. Based on this, it can be assumed that trophoblast cells use the mechanism of suppression of CD105 expression in order to activate VEGF secretion.

Later, we evaluated how treatment of trophoblast cells with antibodies to VEGF affected their survival in the presence of natural killer cells. NK cells successfully killed trophoblast cells, however, treatment with the antibodies did not cause any changes in the resistance of trophoblast cells to the cytotoxic activity of NK cells. The results obtained suggest that VEGF deprivation leads to the previously described effects associated with changes in signal transduction from

TGF-β in trophoblast cells only when they are co-cultured with natural killer cells.

Not only NK cells are able to influence the activity of trophoblast, but trophoblast cells also play a role in placentation by activating various mechanisms that regulate NK cell function [40, 43, 46]. Taking this into account, we evaluated the effect of trophoblast cells and VEGF antibodies on the expression by NK cells of the activating NKG2D receptor (whose ligands are MICA/B molecules), CD94 receptor (whose ligand is the HLA-E molecule expressed by trophoblast), as well as MICA, MICB, and CD105 proteins. The analysis showed that treatment with the antibodies caused a decrease in the expression of CD105 by NK cells in both mono- and co-culture conditions. Since there is no data in the literature on the existence of the SMAD1/5/8 pathway in NK cells, it can be assumed that the treatment with antibodies to VEGF leads to a decrease in the role of endoglin in signaling from TGF-β. As a coreceptor, endoglin not only directs signals from TGF-β, but also regulates the strength of the binding between receptor and other proteins of TGF-β family, such as activin A, BMP-2, BMP-7, BMP-9, and BMP-10, which are also able to affect the cell functions [31].

Treatment with antibodies to VEGF did not affect the expression of the other studied markers by NK cells. Evaluation of the phenotype of NK cells showed that they expressed NKG2D at a high level, both when mono- and co-cultured with trophoblast. Evaluation of the phenotype of NK cells showed that they expressed NKG2D at a high level, both when mono- and co-cultured with trophoblast. In addition, the intensity of expression of this receptor increased upon co-culture conditions, suggesting that NK cells activate the NKG2D-MICA/B pathway when performing cytotoxic functions. As for the CD94 receptor, there was a decrease in the number of cells expressing this receptor following co-culture with trophoblast cells. However, the intensity of its expression increased, which may indicate the differentiation of NK cell populations under the influence of factors secreted by trophoblast cells. This results in the formation of cells that are more sensitive to the HLA-E ligand on the surface of trophoblast cells [9, 29]. With regard to MICA/B markers, it has been observed that their expression in NK cells was increased under co-culture conditions. This is likely due to the cells undergoing a stress response.

Conclusion

Thus, VEGF plays an important role in regulating the activity of trophoblasts and natural killer cells. In particular, the lack of VEGF-A during trophoblast and NK cell co-culture leads to inhibition of CD105 expression by trophoblast cells, which can lead to activation of SMAD2/3 signaling pathways in cells that inhibit cell proliferation. At the same time, treatment of trophoblast cells with antibodies to VEGF for 22 hours did not cause changes in their resistance to the cytotoxic activity of natural killer cells. This suggests that antibodies to VEGF have an inhibitory effect on trophoblast cells only when they are co-cultured with NK cells. In addition, the isolation of VEGF using antibodies caused a decrease in the level of expression of CD105 by NK cells. This indicates that, in the absence of VEGF, the role of this coreceptor in TGF-β signaling decreases. Since the existence of the SMAD1/5/8 pathway in NK cells has not been established, it is possible that in the absence of endoglin, the sensitivity of these cells to other TGF family proteins, such as activin A and BMP-2, BMP-7, BMP-9, and, BMP-10, may change. Together, the results indicate that VEGF deprivation causes significant changes in the reception of TGF-β family proteins by trophoblast cells and natural killer cells. In addition, the data obtained provide an experimental basis for the search for new diagnostic methods in certain forms of obstetric pathology, particularly preeclampsia. In this condition, the assessment of VEGF levels and its functional antagonists, sFlt and endoglin, is of great importance in understanding the pathogenesis of the disease.

Author contributions

Conceptualization, D.S.; methodology, E.T., E.D., O.M.; validation, E.T., D.S.; formal analysis, E.T., D.S.; investigation, E.T.; resources, D.S. and S.S.; data curation, D.S.; writing — original draft preparation, E.T., D.S.; writing — review and editing, S.S.; visualization, E.T., D.S.; supervision, D.S.; project administration, S.S., and D.S.; funding acquisition, I.K., D.S. and S.S. All authors have read and agreed to the published version of the manuscript.

References

1. Ander S.E., Diamond M.S., Coyne C.B. Immune responses at the maternal-fetal interface. *Sci. Immunol., 2019, Vol. 4, no. 31, eaat6114.* doi: 10.1126/sciimmunol.aat6114.

2. Apps R., Gardner L., Traherne J., Male V., Moffett A. Natural-killer cell ligands at the maternal-fetal interface: UL-16 binding proteins, MHC class-I chain related molecules, HLA-F and CD48. *Hum. Reprod., 2008, Vol. 23, no. 11, pp. 2535-2548.*

3. Apte R.S., Chen D.S., Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell, 2019, Vol. 176, no. 6, pp. 1248-1264.*

4. Cabrera-Sharp V., Read J.E., Richardson S., Kowalski A.A., Antczak D.F., Cartwright J.E., Mukherjee A., de Mestre A.M. SMAD1/5 signaling in the early equine placenta regulates trophoblast differentiation and chorionic gonadotropin secretion. *Endocrinology, 2014, Vol. 155, no. 8, pp. 3054-3064.*

5. Chen D.B., Zheng J. Regulation of placental angiogenesis. *Microcirculation, 2014, Vol. 21, no. 1, pp. 15-25.*

6. Chen W.S., Kitson R.P., Goldfarb R.H. Modulation of human NK cell lines by vascular endothelial growth factor and receptor VEGFR-1 (FLT-1). *In Vivo, 2002, Vol. 16, no. 6, pp. 439-445.*

7. Clark D.E., Smith S.K., He Y., Day K.A., Licence D.R., Corps A.N., Lammoglia R., Charnock-Jones D.S. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol. Reprod., 1998, Vol.59, no. 6, pp. 1540-1548.*

8. Clark D.E., Smith S.K., Licence D., Evans A.L., Charnock-Jones D.S. Comparison of expression patterns for placenta growth factor, vascular endothelial growth factor (VEGF), VEGF-B and VEGF-C in the human placenta throughout gestation. *J. Endocrinol., 1998, Vol.159, no. 3, pp. 459-467.*

9. Eidukaite A., Siaurys A., Tamosiunas V. Differential expression of KIR/NKAT2 and CD94 molecules on decidual and peripheral blood CD56bright and CD56dim natural killer cell subsets. *Fertil. Steril., 2004, Vol. 81 Suppl. 1, pp. 863-868.*

10. Fitzpatrick T.E., Lash G.E., Yanaihara A., Charnock-Jones D.S., Macdonald-Goodfellow S.K.,Graham C.H. Inhibition of breast carcinoma and trophoblast cell invasiveness by vascular endothelial growth factor. *Exp. Cell Res., 2003, Vol. 283, no. 2, pp. 247-255.*

11. Fong G.H., Rossant J., Gertsenstein M., Breitman M.L. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature, 1995, Vol. 376, no. 6535, pp. 66-70.*

12. Garcia J., Hurwitz H.I., Sandler A.B., Miles D., Coleman R.L., Deurloo R., Chinot O.L. Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and future outlook. *Cancer Treat. Rev., 2020, Vol. 86, 102017.* doi: 10.1016/j.ctrv.2020.102017.

13. Gong J.H., Maki G., Klingemann H.G. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. *Leukemia, 1994, Vol. 8, no. 4, pp. 652-658.*

14. Guo B., Slevin M., Li C., Parameshwar S., Liu D., Kumar P., Bernabeu C., Kumar S. CD105 inhibits transforming growth factor-beta-Smad3 signalling. *Anticancer Res., 2004, Vol.24, no. 3a, pp. 1337-1345.*

15. Haider S., Lackner A.I., Dietrich B., Kunihs V., Haslinger P., Meinhardt G., Maxian T., Saleh L., Fiala C., Pollheimer J., Latos P.A., Knofler M. Transforming growth factor-beta signaling governs the differentiation program of extravillous trophoblasts in the developing human placenta. *Proc. Natl. Acad. Sci. U S A, 2022, Vol. 119, no. 28, e2120667119.* doi: 10.1073/pnas.2120667119.

16. Hurwitz H.I., Fehrenbacher L., Hainsworth J.D., Heim W., Berlin J., Holmgren E., Hambleton J., Novotny W.F., Kabbinavar F. Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. *J. Clin. Oncol., 2005, Vol. 23, no. 15, pp. 3502-3508.*

17. Ito N., Wernstedt C., Engstrom U., Claesson-Welsh L. Identification of vascular endothelial growth factor receptor-1 tyrosine phosphorylation sites and binding of SH2 domain-containing molecules. *J. Biol. Chem., 1998, Vol. 273, no. 36, pp. 23410-23418.*

18. Jackson M.R., Carney E.W., Lye S.J., Ritchie J.W. Localization of two angiogenic growth factors (PDECGF and VEGF) in human placentae throughout gestation. *Placenta, 1994, Vol.15, no. 4, pp. 341-353.*

19. Jin X., Mao L., Zhao W., Liu L., Li Y., Li D., Zhang Y., Du M. Decidualization-derived cAMP promotes decidual NK cells to be angiogenic phenotype. *Am. J. Reprod. Immunol., 2022, Vol.88, no. 3, e13540.* doi: 10.1111/aji.13540.

20. Kendall R.L., Wang G., Thomas K.A. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. *Biochem. Biophys. Res. Commun., 1996, Vol. 226, no. 2, pp. 324-328.*

21. Kohler P.O., Bridson W.E. Isolation of hormone-producing clonal lines of human choriocarcinoma. *J. Clin. Endocrinol. Metab., 1971, Vol.32, no. 5, pp. 683-687.*

22. Laakkonen J.P., Lahteenvuo J., Jauhiainen S., Heikura T., Yla-Herttuala S. Beyond endothelial cells: Vascular endothelial growth factors in heart, vascular anomalies and placenta. *Vascul. Pharmacol., 2019, Vol. 112, pp. 91-101.*

23. Lebrin F., Goumans M.J., Jonker L., Carvalho R.L., Valdimarsdottir G., Thorikay M., Mummery C., Arthur H.M., ten Dijke P. Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *EMBO J., 2004, Vol. 23, no. 20, pp. 4018-4028.*

24. Li D.Y., Sorensen L.K., Brooke B.S., Urness L.D., Davis E.C., Taylor D.G., Boak B.B., Wendel D.P. Defective angiogenesis in mice lacking endoglin. *Science, 1999, Vol. 284, no. 5419, pp. 1534-1537.*

25. Li Y., Zhu H., Klausen C., Peng B., Leung P.C. Vascular Endothelial Growth Factor-A (VEGF-A) Mediates Activin A-Induced Human Trophoblast Endothelial-Like Tube Formation. *Endocrinology, 2015, Vol. 156, no. 11, pp. 4257-4268.*

26. Maynard S.E., Min J.Y., Merchan J., Lim K.H., Li J., Mondal S., Libermann T.A., Morgan J.P., Sellke F.W., Stillman I.E., Epstein F.H., Sukhatme V.P., Karumanchi S.A. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J. Clin. Invest., 2003, Vol.111, no. 5, pp. 649-658.*

27. Melder R.J., Koenig G.C., Witwer B.P., Safabakhsh N., Munn L.L., Jain R.K. During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. *Nat. Med., 1996, Vol. 2, no. 9, pp. 992-997.*

28. Melincovici C.S., Bosca A.B., Susman S., Marginean M., Mihu C., Istrate M., Moldovan I.M., Roman A.L., Mihu C.M. Vascular endothelial growth factor (VEGF) – key factor in normal and pathological angiogenesis. *Rom. J. Morphol. Embryol., 2018, Vol. 59, no. 2, pp. 455-467.*

29. Mikhailova V., Khokhlova E., Grebenkina P., Salloum Z., Nikolaenkov I., Markova K., Davidova A., Selkov S., Sokolov D. NK-92 cells change their phenotype and function when cocultured with IL-15, IL-18 and trophoblast cells. *Immunobiology, 2021, Vol. 226, no. 5, 152125*. doi: 10.1016/j.imbio.2021.152125.

30. Naderan M., Sabzevary M., Rezaii K., Banafshehafshan A., Hantoushzadeh S. Intravitreal anti-vascular endothelial growth factor medications during pregnancy: current perspective. *Int. Ophthalmol., 2021, Vol. 41, no. 2, pp. 743-751.*

31. Nickel J., Ten Dijke P.,Mueller T.D. TGF-beta family co-receptor function and signaling. *Acta Biochim. Biophys. Sin., 2018, Vol. 50, no. 1, pp. 12-36.*

32. Papadopoulos N., Martin J., Ruan Q., Rafique A., Rosconi M.P., Shi E., Pyles E.A., Yancopoulos G.D., Stahl N., Wiegand S.J. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. *Angiogenesis, 2012, Vol. 15, no. 2, pp. 171-185.*

33. Rajagopalan S., Long E.O. KIR2DL4 (CD158d): An activation receptor for HLA-G. *Front. Immunol., 2012, Vol. 3, 258.* doi: 10.3389/fimmu.2012.00258.

34. Sandler A., Gray R., Perry M.C., Brahmer J., Schiller J.H., Dowlati A., Lilenbaum R., Johnson D.H. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N. Engl. J. Med., 2006, Vol. 355, no. 24, pp. 2542-2550.*

35. Scherner O., Meurer S.K., Tihaa L., Gressner A.M., Weiskirchen R. Endoglin differentially modulates antagonistic transforming growth factor-beta1 and BMP-7 signaling. *J. Biol. Chem., 2007, Vol.282, no. 19, pp. 13934-13943.*

36. Schiessl B., Innes B.A., Bulmer J.N., Otun H.A., Chadwick T.J., Robson S.C., Lash G.E. Localization of angiogenic growth factors and their receptors in the human placental bed throughout normal human pregnancy. *Placenta, 2009, Vol. 30, no. 1, pp. 79-87.*

37. Sharkey A.M., Charnock-Jones D.S., Boocock C.A., Brown K.D.,Smith S.K. Expression of mRNA for vascular endothelial growth factor in human placenta. *J. Reprod. Fertil., 1993, Vol. 99, no. 2, pp. 609-615.*

38. Sharma S., Godbole G.,Modi D. Decidual Control of Trophoblast Invasion. *Am. J. Reprod. Immunol., 2016, Vol. 75, no. 3, pp. 341-350.*

39. Simons M., Gordon E., Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. Nat. Rev. Mol. *Cell Biol., 2016, Vol. 17, no. 10, pp. 611-625.*

40. Tan H.X., Yang S.L., Li M.Q., Wang H.Y. Autophagy suppression of trophoblast cells induces pregnancy loss by activating decidual NK cytotoxicity and inhibiting trophoblast invasion. *Cell Commun. Signal., 2020, Vol. 18, no. 1, 73.* doi: 10.1186/s12964-020-00579-w.

41. Trembath A.P., Markiewicz M.A. More than Decoration: roles for natural killer group 2 member d ligand expression by immune cells. *Front. Immunol., 2018, Vol. 9, 231.* doi: 10.3389/fimmu.2018.00231.

42. Vinnars M.T., Bjork E., Nagaev I., Ottander U., Bremme K., Holmlund U., Sverremark-Ekstrom E., Mincheva-Nilsson L. Enhanced Th1 and inflammatory mRNA responses upregulate NK cell cytotoxicity and NKG2D ligand expression in human pre-eclamptic placenta and target it for NK cell attack. *Am. J. Reprod. Immunol., 2018, Vol. 80, no. 1, e12969.* doi: 10.1111/aji.12969.

43. Wallace A.E., Fraser R., Cartwright J.E. Extravillous trophoblast and decidual natural killer cells: a remodelling partnership. *Hum. Reprod. Update, 2012, Vol. 18, no. 4, pp. 458-471.*

44. Waltenberger J., Claesson-Welsh L., Siegbahn A., Shibuya M., Heldin C.H. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J. Biol. Chem., 1994, Vol. 269, no. 43, pp. 26988-26995.*

45. Wang J., Ding J., Zhang S., Chen X., Yan S., Zhang Y., Yin T. Decreased USP2a Expression Inhibits Trophoblast Invasion and Associates With Recurrent Miscarriage. *Front. Immunol., 2021, Vol. 12, 17370.* doi: 10.3389/fimmu.2021.717370.

46. Wang X.Q., Zhou W.J., Hou X.X., Fu Q., Li D.J. Trophoblast-derived CXCL16 induces M2 macrophage polarization that in turn inactivates NK cells at the maternal-fetal interface. *Cell. Mol. Immunol., 2018, Vol. 15, no. 12, pp. 1038-1046.*

47. Wu D., Luo S., Wang Y., Zhuang L., Chen Y., Peng C. Smads in human trophoblast cells: expression, regulation and role in TGF-beta-induced transcriptional activity. *Mol. Cell. Endocrinol., 2001, Vol. 175, no. 1-2, pp. 111-121.*

48. Yang F., Zheng Q., Jin L. Dynamic Function and Composition Changes of Immune Cells During Normal and Pathological Pregnancy at the Maternal-Fetal Interface. *Front. Immunol., 2019, Vol. 10, 2317.* doi: 10.3389/fimmu.2019.02317.

49. Yi Y., Cheng J.C., Klausen C., Leung P.C.K. TGF-beta1 inhibits human trophoblast cell invasion by upregulating cyclooxygenase-2. *Placenta, 2018, Vol. 68, pp. 44-51.*

50. Zhao J., Schlosser H.A., Wang Z., Qin J., Li J., Popp F., Popp M.C., Alakus H., Chon S.H., Hansen H.P., Neiss W.F., Jauch K.W., Bruns C.J., Zhao Y. Tumor-derived extracellular vesicles inhibit natural killer cell function in pancreatic cancer. *Cancers, 2019, Vol. 11, no. 6, 874.* doi: 10.3390/cancers11060874.

51. Zhou Y., McMaster M., Woo K., Janatpour M., Perry J., Karpanen T., Alitalo K., Damsky C., Fisher S.J. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am. J. Pathol., 2002, Vol. 160, no. 4, pp. 1405-1423.*

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Поступила 23.04.2024
Принята к печати 14.09.2024

Received 23.04.2024
Accepted 14.09.2024