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THE ROLE OF NEUROPILIN-1 (NRP1) IN THE DEVELOPMENT OF SARS-COV-2 INFECTION

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A review of the literature on the role of neuropilin-1 in the development of SARS-CoV-2 infection and a search for probable links between polymorphic variants of the *NRP1* gene and SARS-CoV-2 are presented. This review presents the characteristics of polymorphic variants of the *NRP1* gene, which demonstrate the possibility of their association with the course of SARS-CoV-2 infection.

Keywords: SARS-CoV-2 infection, neuropilin-1, polymorphic variants of the *NRP1* gene.

Introduction. With the onset of the SARS-CoV-2 coronavirus infection pandemic, it was necessary to understand the mechanisms of penetration of this pathogen into the cell and the mechanisms of their interaction as early as possible. In 2020, it was found that the furin-cleaved S1 fragment of the SARS-CoV-2 spike protein directly binds to cell surface neuropilin-1 [6].

Neuropilin-1 (NRP1) is a transmembrane glycoprotein. The neuropilin-1 receptor plays a key role in the development of the nervous and vascular systems, as neuropilins mediate VEGF (vascular endothelial growth factor) dependent angiogenesis and semaphorin-dependent axonal growth direction. In addition, the participation of neuropilins in a wide variety of signaling and adhesive functions has been studied, which indicates their high role as pleiotropic coreceptors [12].

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NRP1 consists of 923 amino acids and has a massive extracellular portion that includes two tandem CUB domains (a1/a2), two tandem domains homologous to coagulation factors V/VIII (b1/b2), a linker sequence, and one MAM domain (C) that supports dimerization and multimerization of neuropilin molecules and promotes the formation of signal receptor complexes [27]. The cytoplasmic domain, which includes 44 amino acid residues, contains a sequence of three C-terminal amino acid residues (SEA-COOH) and demonstrates high phylogenetic conservatism [30].

Neuropilin-1 promotes the breakdown of the spike protein. Cleavage of the SARS-CoV-2 S protein at the S1-S2 site results in the C-terminal sequence TQTNSPRRAR-OH. AgNP nanoparticles coated with the TQTNSPRRAR-OH peptide sequence were efficiently taken up by the neuropilin-positive cell culture. Intensive uptake of AgNP-TQTNSPRRAR-OH by the olfactory epithelium, neurons, and blood vessels of the cerebral cortex has also been shown [6]. NRP1 can modulate SARS-CoV-2 infection by stimulating the separation of the S1 and S2 subunits. Additional sites of interaction between neuropilin-1 and the spike protein, which function as additional points of connection with the lipid bilayer of the infected cell, play a significant role [21]. In turn, the results of isothermal titration calorimetry demonstrate a direct relationship between the b1 domain of NRP1 and the synthetic S1 peptide (679-NSPRRAR-685) with an affinity of 20.3 μM at pH 7.5, and this crystal structure showed significant similarity [7] with the crystal structure b1 domain of NRP1 in complex with its endogenous VEGF-A ligand [28].

Functional and structural diversity of binding sites for neuropilin-1 and spike protein. The analysis of interaction sites between SARS-CoV-2 S-protein and human neuropilin-1 deserves special attention: amino acid residues GLN280, ASP289, TYR322, ARG323, TRP325, GLN327, ASP329, LYS359, ASP361 have been identified as potential binding sites in the b1 domain of NRP1. Relationships are also observed between GLN3, ILE8, PHE29, ALA30 RBD of SARS-CoV-2 S-protein domain and ARG402, ARG405, LYS407 of NRP1 b1 domain [2]. The overlap of SARS-CoV-2 RBD checkpoints with the VEGF-associated NRP1 site is confirmed, and interaction with GLN280 can serve as an example [18]. In turn, the amino acid residues TYR322, ARG323, TRP325, GLN327, ASP329, LYS359, ASP361 are structurally close to the VEGF-binding site of NRP1; moreover, TYR297, ASP320, SER346, THR349, TYR353 play a leading role in its structure [33]. All this indi-

cates that the binding of the SARS-CoV-2 S protein to the b1 domain of NRP1 suppresses the binding of VEGF to neuropilin-1, which was shown by blocking the VEGF-mediated increase in the activation of calcium and sodium channels [24]. Interestingly, the SARS-CoV-2 S-protein RBD-domain (RBD) binds to LYS359 and ASP361 [2], located in the sequence of the neuropilin-1 adhesion site (347-364) [33]. It should be noted that the receptor-binding domain of the SARS-CoV-2 S-protein binds to the positively charged conserved amino acid LYS/ARG359 [2], which is necessary for the binding of NRP1 and heparin [33], which plays a significant role in the course and therapy of COVID-19. [fifteen]. The amino acid residues of THR316, PRO317, and ASP320, as well as SER346, THR349, and TYR353 of NRP1, form relatively strong H-bonds with the positively charged guanidine group and the negatively charged carboxyl portion S1 of the SARS-CoV-2 protein. The guanidine group of ARG685 forms an electrostatic bond with the negatively charged ASP320 and forms hydrogen bonds with the THR316, PRO317, and ASP320 residues of neuropilin-1, while its C-terminal carboxyl group forms hydrogen bonds with SER346, THR349, and TYR353 of NRP1 [20].

It is important to note that the conditional boundary between the S1 and S2 domains runs between amino acid residues 685 (S1) and 686 (S2) of the SARS-CoV-2 spike protein [21], while the main site of interaction between the S1 protein and neuropilin-1 begins at 678 /682 [7; 24] position and ends at position 685 [7]. Moreover, it is arginine at position 685 that is critical for SARS-CoV-2 S1 association with neuropilin-1 through electrostatic interactions with the negatively charged aspartic acid at position 320 and forms hydrogen bonds with THR316, PRO317, and ASP320 amino acids [20]. This destabilizes several basic interactions between S1 and S2. The destabilization of the link between the RBD domain of the S1 protein and the 686-1146 sequence of the S2 protein is mediated by the interaction of RBD with ACE2. However, the cleavage site at position 685/686 is still provided by S1 and S2 binding. However, binding of the 682-RRAR-685 motif to NRP1 at the cleavage site provides accelerated S2 separation, increasing virus infectivity [21].

Peculiarities of NRP1 expression during SARS-CoV-2 infection. Although NRP1 does not by itself mediate infection in cell culture, its co-expression with ACE2 and TMPRSS2 markedly enhances infectivity. With isolated expression of *NRP1*, lower levels of symptomatic infection load were observed [6].

A post-mortem study of 2 patients with anosmia showed focal atrophy of the olfactory epithelium, leukocyte infiltration of the *lamina propria*, and signs of axonal damage to the olfactory nerve fibers [19]. Thus, the extensive role of NRP1 in the immunosuppressive function of regulatory T cells [5], extensive damage to the lungs, olfactory epithelial cells, and olfactory sensory neurons may be related [13].

The lungs of COVID-19 patients show characteristic vascular features. Histological analysis of pulmonary vessels in patients with COVID-19 showed severe thrombosis with microangiopathy. For example, microthrombi in the alveolar capillaries were 9 times more common in patients with COVID-19 than in patients with influenza, and in the lungs of patients with COVID-19, the number of new vessels growing mainly through invagination angiogenesis was 2.7 times higher than in the lungs of patients. influenza [1]. By controlling endothelial adhesion and permeability, NRP1 may be involved in pathological coagulation. By binding the b1 domain of NRP1 and thereby blocking traditional angiogenic ligands, SARS-CoV-2 may contribute to vascular dysfunction and coagulation throughout the body [25].

It should be emphasized that *ACE2* and *TMPRSS2* have a relatively lower level of expression in the CNS [14], therefore, most of the symptoms of COVID-19 in the CNS are usually attributed to the consequences of damage to the peripheral systems of the body [3]. However, convincing evidence has been presented that the virus can infect cells via neuropilin-1 [6;7]. Several studies have shown that infected vascular endothelial cells mediate the spread of SARS-CoV-2 to glial cells in the central nervous system. Thus, neuropilin-1 has been proposed as a key factor in a wide range of neurological manifestations of COVID-19 by increasing the penetration of SARS-CoV-2 into the brain [4].

Significance of neuropilin-1 for the immune response to SARS-CoV-2. Neuropilin-1 has a strong effect on virus-induced production of INF-α by dendritic cells [31] a twofold lower virus-induced production of IFN-α was shown in anti-NRP1-treated dendritic cells compared to untreated dendritic cells [11]. Thus, NRP1 may increase the susceptibility of dendritic cells to SARS-CoV-2 infection by mediating the internalization of the virus into uninfected cells, followed by the production and secretion of cytokines, which can lead to a cytokine storm and an increased risk of complications [31].

The researchers highlight the ability of the CD25+ CD4+ Foxp3+ subset of regulatory T cells to significantly influence the immunological balance in COVID-19. SARS-CoV-2 infection induces the transcription of IL-2, which binds to soluble CD25 in the blood, leading to CD28+ CD4+ mediated release of pro-inflammatory cytokines [9].

Association of polymorphic variants of the *NRP1* gene with multifactorial diseases: the search for probable links with SARS-CoV-2. Based on the above data, we can assume the functional significance of polymorphic variants of the *NRP1* gene for the development, course, and outcome of SARS-CoV-2 infection in humans. For example, it has been reported that the rs10080 G>A polymorphism is associated with reduced *NRP1* expression, and individuals carrying the *G* allele may express lower levels of neuropilin-1 in target cells, which may influence the neuropathogenesis associated with COVID-19 disease [16].

An association of the polymorphic variant *rs2506142* (minor allele *G*) of the *NRP1* gene with the risk of developing standard and menstrual migraine has been demonstrated [29]. In the development of migraine, the regulation of the concentration of cytosolic calcium ions is of particular importance [35], and the VEGF-A mediated effect of NRP1 on nociceptive activation is expressed precisely in an increase in the total number of sodium and calcium channels in the neurons of the spinal ganglia. In turn, the SARS-CoV-2 S protein inhibits pronociceptive VEGF-A/NRP-1 signaling and has an analgesic effect in chronic neuropathic pain in rats [24].

Several studies reveal a significant role of neuropilin-1 in the pathogenesis of malignant neoplasms. Relatively high levels of *NRP1* expression were observed in squamous cell carcinoma of the kidney, hepatocellular liver cancer, thyroid cancer, and gastric adenocarcinoma; however, researchers note the involvement of neuropilin-1 in pathological angiogenesis as the leading mechanism of pathogenesis [22]. In this context, it is important to recall that in the lungs of patients with COVID-19, there is an active growth of new vessels, mainly through the mechanism of invagination angiogenesis [1].

The *rs2228638* polymorphic variant is of interest in the context of its influence on the relationship between neuropilin-1 and SARS-CoV-2, since the SARS-

CoV-2 S protein competes with VEGF-A for interaction with neuropilin-1 [17; 24]. It has been shown that this polymorphic variant is associated with several cardiovascular anomalies, and the main reason for this association is a decrease in the activity of NRP1 as a coreceptor in VEGF intermolecular signaling [8].

The *GA* and *AA* genotypes of the *rs2070296* polymorphic locus are associated with a weaker response to antiangiogenic therapy via ranibizumab blockade of VEGF-A [23]. It is also important to report the ability of the *rs3750733 C/T* polymorphic variant of the *NRP1* gene to modulate VEGF-dependent angiogenesis [10].

For the group of polymorphic variants of the *NRP1* gene: *rs750880625 c.676C>T* p. R226C; *rs180868035 c.A418C* p.I140L; *rs1178713109 c.A1274T* p.K425M; *rs117525057 c.C1571T* p.S524L; *rs143124682 c.C1676T* p.T559M; *rs767902777 c.2200G>A* p.G734S; *rs548175518 c.2596G>A* p.A866T and *rs566437913 c.T2633C* p.V878A showed an association with idiopathic hypogonadotropic hypogonadism (IHH; English Idiopathic hypogonadotropic hypogonadism) associated with impaired sense of smell (Kallman syndrome) [26]. Neuropilin-1 has also been shown to be significantly more expressed than ACE2 in the olfactory epithelium [6] and may play a central role in olfactory dysfunction during SARS-CoV-2 infection [13]. *NRP1* is expressed along the vomeronasal/terminal nerve pathway and is involved in the migration of gonadotropin releasing neurons (GnRH neurons) [26].

These data are of extremely high interest in the context of the impact of SARS-CoV-2 infection on the reproductive capacity of men. For example, a significant pathological effect of coronavirus infection on reproductive ability, mediated by the development of orchitis, has been shown. However, no traces of the presence of the virus were found in the testicles [34]. Changes in sperm parameters and the level of sex hormones were also shown, in turn, disturbances in the homeostasis of hormones of the pituitary-testicular axis are isolated as one of the possible pathological mechanisms of impaired fertility during SARS-CoV-2 infection [32].

Conclusion. Neuropilin-1 has been shown to modulate SARS-CoV-2 infection by playing a leading role in separating the S1 and S2 subunits of the spike protein. Several studies demonstrate the significant role of neuropilin-1 in the immune response. Researchers note its significant role in pathological phenomena from the vascular system and the central nervous system. All this points to the need for further research on the role of NRP1 in the development of SARS-CoV-2 infection.

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POINT OF VIEW

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DETECTION OF BETA-GLOBIN GENES IN THE ERYTHROCYTE FRACTION IN A PATIENT WITH CERVICAL CANCER

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The results of detection of DNA fragments encoding human beta-globin and human papillomavirus (HPV) L1 protein in cervical cancer patient's plasma and erythrocyte samples using real-time polymerase chain reaction (qPCR) are presented. Amplification of a DNA fragment encoding human beta-globin was found only in a sample of erythrocytes, which may indirectly indicate the presence of extracellular DNA. The results of qPCR for the presence of a DNA fragment encoding HPV L1 were negative, which did not allow us to confirm that the isolated DNA belonged to the tumor DNA. The origin of the identified in the erythrocyte fraction DNA fragment encoding human beta-globin requires further research.

Keywords: tumor DNA, extracellular vesicles, neutrophil extracellular traps.

Beta-globin is a part of hemoglobin - an iron-containing metalloprotein. In mammals hemoglobin is found in the largest amount in red blood cells, its main function is to transport oxygen from the lungs to various body tissues. Hemoglobin also interacts with other gases such as carbon dioxide, carbon monoxide, and nitric oxide. Hemoglobin is a tetramer composed of two beta-globin chains and also of two alpha-globin chains. Heme is attached to each of these chains, and thus each chain can transport oxygen. Hemoglobin is expressed by erythroid cells as well as by nonerythroid cells, such as epithelial cells, including epithelial cells of the cervix uteri [22].

It has been established that in normal cells of the cervical and vaginal mucosa alpha-globin and beta-globin can act as endogenous antimicrobial protective proteins against infection [21]. Hemoglobin and mRNA are also found in human cervical carcinoma cell lines (SiHa and CaSki), and the expression of alpha-globin and beta-globin genes in them is significantly higher than in normal cells of the cervix uteri [14]. Expression of these genes improves the viability of tumor cells by suppressing oxidative stress [28].

Alpha-globin and beta-globin are encoded by genes located on chromosomes 16 and 11, respectively. The beta-globin gene cluster is packed into inactive heterochromatin in non-erythroid cells, while the alpha-globin genes are built into open chromatin conformations in all cell types [9]. Transcriptionally inactive heterochromatin plays a vital role in maintaining a stable structure of specialized chromosomal regions with repetitive DNA; loss of integrity in these regions of chromosomes can contribute to the cancer development [7].

DNA, including genomic DNA, is found in blood fractions purified from cells - in plasma and serum. Both normal and tumor cells release DNA into the circulation, but it is present in increased amounts in cancer patients [23]. It is assumed that the detection of tumor DNA in the blood can significantly improve the detection of a tumor at an early stage, determine its progression and prognosis, and also help in targeted therapy [5]. Detection of tumor DNA is carried out mainly in blood plasma, but the complexity of the determination is associated with low concentration of DNA in plasma [11].

Human genomic DNA is also found in the erythrocyte fraction of blood, even after 25 years of storage [4]. Genomic DNA, both of human and infectious, can be associated with the surface of erythrocytes through receptors, since it is known that erythrocytes express receptors on their surface, which, at least bind bacterial and mitochondrial DNA in vitro [13].

We assumed that in cervical cancer (CC) patient genomic DNA is contained in the blood in an increased amount, and the presence of beta-globin genes in genomic DNA can be an indicator of carcinogenesis, so the goal of the study was to isolate DNA from the patient's blood fractions in which there are no nuclear cells - from plasma and erythrocyte fraction, as well as to detect a DNA fragment encoding human beta-globin. In order to determine, let indirectly, whether the isolated DNA belongs to a tumor DNA, it was decided to detect a DNA fragment encoding the L1 protein region of the human papillomavirus (HPV). Since it is believed, that in CC patient circulating extracellular DNA containing the HPV genome originates from tumor cells [8].

Materials and methods. 4 types of biological samples were prepared from venous blood taken by venipuncture in vacuum blood collection tubes-K3 EDTA: plasma samples (sample 1); a suspen-

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