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REVIEWS

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REVIEW
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Exosomes in the life cycle of viruses and the pathogenesis of viral infections

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Exosomes are extracellular vesicles of endosomal origin, with a bilayer membrane, 30–160 nm in diameter. Exosomes are released from cells of different origins and are detected in various body fluids. They contain nucleic acids, proteins, lipids, metabolites and can transfer the contents to recipient cells. Exosome biogenesis involves cellular proteins of the Rab GTPase family and the ESCRT system, which regulate budding, vesicle transport, molecule sorting, membrane fusion, formation of multivesicular bodies and exosome secretion. Exosomes are released from cells infected with viruses and may contain viral DNA and RNA, as well as mRNA, microRNA, other types of RNA, proteins and virions. Exosomes are capable of transferring viral components into uninfected cells of various organs and tissues. This review analyzes the impact of exosomes on the life cycle of widespread viruses that cause serious human diseases: human immunodeficiency virus (HIV-1), hepatitis B virus, hepatitis C virus, SARS-CoV-2. Viruses are able to enter cells by endocytosis, use molecular and cellular pathways involving Rab and ESCRT proteins to release exosomes and spread viral infections. It has been shown that exosomes can have multidirectional effects on the pathogenesis of viral infections, suppressing or enhancing the course of diseases. Exosomes can potentially be used in noninvasive diagnostics as biomarkers of the stage of infection, and exosomes loaded with biomolecules and drugs – as therapeutic agents. Genetically modified exosomes are promising candidates for new antiviral vaccines.

Keywords: *review; exosomes; extracellular vesicles; endocytosis; Rab GTPases; ESCRT system; exocytosis; human immunodeficiency virus; hepatitis B virus; hepatitis C virus; SARS-CoV-2*

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Экзосомы в жизненном цикле вирусов и патогенезе вирусных инфекций

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Экзосомы – внеклеточные везикулы эндосомального происхождения с двухслойной мембраной, диаметром 30–160 нм. Экзосомы высвобождаются из клеток разного происхождения и определяются в различных биологических жидкостях организма. Они содержат клеточные нуклеиновые кислоты, белки, липиды, метаболиты и могут передавать содержимое клеткам-реципиентам. В биогенезе экзосом участвуют клеточные белки семейства Rab ГТФаз и системы ESCRT, которые регулируют почкование, транспорт везикул, сортировку молекул, слияние мембран, образование мультивезикулярных телец и секрецию экзосом. Из клеток, инфицированных вирусами, высвобождаются экзосомы, которые содержат геномные вирусные ДНК и РНК, а также мРНК, микроРНК, другие виды РНК, белки и вирионы. Экзосомы способны переносить вирусные компоненты в неинфицированные клетки различных органов и тканей. В настоящем обзоре проанализировано влияние экзосом на жизненный цикл широко распространённых вирусов, вызывающих серьёзные заболевания человека: вирус иммунодефицита человека 1-го типа, вирус гепатита В, вирус гепатита С, SARS-CoV-2. Вирусы способны проникать в клетки путём эндоцитоза, используют молекулярные и клеточные пути с участием белков Rab и ESCRT для высвобождения экзосом и распространения вирусных инфекций. Показано, что экзосомы могут оказывать разнонаправленные действия на патогенез вирусных инфекций, подавляя или способствуя развитию вызываемых ими заболеваний. Экзосомы потенциально могут использоваться в неинвазивной диагностике как биомаркеры стадии инфекции, а экзосомы, нагруженные биомолекулами и лекарственными препаратами, – как терапевтические средства. Генетически модифицированные экзосомы – перспективные кандидаты для новых противовирусных вакцин.

Ключевые слова: *обзор; экзосомы; внеклеточные везикулы; эндоцитоз; Rab ГТФазы; система ESCRT; экзоцитоз; вирус иммунодефицита человека; вирус гепатита В; вирус гепатита С; SARS-CoV-2*

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Introduction

Exosomes were first identified in the culture medium of reticulocytes. In 1983, almost at the same time, two research groups reported that in reticulocytes, transferrin receptors associated with intracellular vesicles (approximately 50 nm diameter-sized) were literally jettisoned from maturing reticulocytes into the extracellular space [1, 2]. Harding et al. [1, 3] found that in cells, internalized transferrin is located on numerous small particles within organelles, which the authors referred to as multivesicular endosomes. Multivesicular endosomes fuse with the plasma membrane, leading to release (externalization) of vesicles later renamed as exosomes [4]. It was assumed that cells could use that mechanism as a universal process of release of membrane vesicles. The discovery was met with great interest among researchers, leading to hundreds of publications on exosomes, the launch of the scientific Journal of Extracellular Vesicles, the foundation of specialty societies: The International Society for Extracellular Vesicles, The American Society for Exosomes and Microvesicles.

The review briefly touches upon the aspects of the biogenesis and functions of exosomes. Special attention is given to the role of exosomes in viral infections.

Characterization of exosomes

Exosomes are extracellular vesicles (EVs) with a double-layer membrane structure, a diameter of 30–160 nm (around 100 nm on average), and an endosomal origin. The most common markers for exosomes include CD63, CD81 and CD9, flotillins, ceramides, TSG101 (tumor susceptibility gene 101) and Alix (apoptosis-linked gene 2 interacting protein X) [5, 6] **(Fig. 1)**. Exosomes have been found to contain lipids, proteins, all known RNA species, metabolites [7, 8]. After release from the cell surface, exosomes can interact with the extracellular matrix and (or) enter into recipient cells [9]. Exosomes are released by cells of different origin; they are found in various body fluids: in blood plasma and serum, urine, cerebrospinal fluid, breast milk, saliva, gastric acid, semen and follicular fluid, and in feces [7, 10, 11]. These vesicles are characterized by high heterogeneity in terms of size and functions [12]. The content (cargo) of exosomes varies and depends on the cellular origin, metabolic status and environment of donor cells; therefore, there are multiple subgroups of exosomes [8]. By transporting various bioactive molecules to recipient cells, exosomes can participate in regulation of transcription and translation; proliferation, reproduction, growth, cell differentiation, pathological processes, including neoplasia [12, 13].

Improvement of the techniques used for isolation and purification of vesicles is one of the major challenges in exosome research and application. In addition to ultracentrifugation, density gradient centrifugation and ultrafiltration, more advanced methods and tools have been developed: the exosome total isolation chip (ExoTIC) [14], the asymmetric-flow field-flow fractionation (AF4) technology [15], the exosome-specific dual-patterned immunofiltration (ExoDIF) device [16]. Recent methods used for exosome identification, size and content evaluation employ electron microscopy and flow cytometry [5]. The frequency of using nine different methods for isolation and purification of exosomes was assessed in 2019 and showed that studies employing immunoaffinity techniques, fluorescence-activated cell sorting (FACS) and polymer-based precipitation accounted approximately for 3–5%, density gradient centrifugation – 25%, filtration – 34%, ultracentrifugation – 58% and differential centrifugation – 73% of the total number of all the analyzed publications. While differential centrifugation remains the most commonly used method, it has been noted that most studies have employed a combination of these methods [17]. Further improvement of exosome isolation and purification methods is required for reliable classification of exosomes and standardization of their application. EVs can be divided into three main types depending on their cellular origin, density ranges, expression of markers and size:

a) exosomes (30–150 nm);
b) microvesicles (100–1000 nm);
c) apoptotic bodies (500–5000 nm) [18].

Significant variations in the size of vesicles should be taken into consideration [19]. In 2018, experts from The International Society for Extracellular Vesicles published guidelines recommending the term “extracellular vesicles” or “EVs” as the most accurate term for the cases when the biogenesis and distinct properties of the vesicles had not been studied in detail, and they significantly varied in size. The term “exosomes” is used to refer to small EVs with diameter not larger than 200 nm [20]. However, the purity of vesicles was not assessed in most studies, and populations of vesicles could contain both exosomes and microvesicles. Therefore, in our further discussion of findings, we will use the terminology offered in original publications.

Exosome biogenesis

After components of the external environment penetrate the cell by endocytosis, the internalized material is delivered to early endosomes (EEs) **(Fig. 2)**. The subsequent maturation of EEs induces a decrease in pH inside the organelle, accumulation of phosphatidylinositol-3-phosphate (PI3P) in the membrane, involvement of some enzymes of the Rab family and their activation. This is followed by the formation of late endosomes (LEs). Like EEs, LEs perform cargo sorting as well as sensing and signaling functions, responding to inter- and extracellular situations [21]. The endosomal membrane bends inwards to generate multiple intraluminal vesicles (ILVs), which form multivesicular bodies (MVBs).

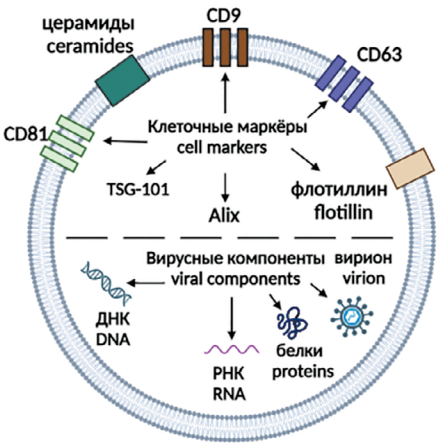

Fig. 1. Exosome biomarkers and content.

In the upper half are the main markers of exosomes: membrane proteins – tetraspanins CD9, CD63, CD81 and flotillins; lipids – ceramides, components of the ESCRT system Alix и TSG101. In the lower half – viral components that are captured by exosomes in infected cells – DNA or RNA, viral structural and non-structural proteins and virions.

Рис. 1. Содержимое экзосом и биомаркеры.

В верхней половине – основные маркеры экзосом, представленные мембранными белками – тераспанинами CD9, CD63, CD81 и флотиллинами; липидами – церамидами, а также компонентами клеточной системы ESCRT (Endosomal Sorting Complex Required for Transport): Alix (apoptosis-linked gene 2 interacting protein X) и TSG101 – продукт Tumor Susceptibility Gene 101 (*tsg101*). В нижней половине – вирусные компоненты, которые захватываются экзосомами в заражённых клетках, – геномные ДНК или РНК, вирусные структурные и неструктурные белки и вирионы.

From MVBs, internalized materials incorporated into ILVs are transported along one of the three possible pathways (Fig. 2): The first one – to the recycling endosomes for processing; then to the plasma membrane or to the Golgi complex; the second one – to the lysosome for fusion with the lysosome and subsequent degradation; the third one – to the plasma membrane for fusion followed by the release in the form of exosomes into the extracellular space.

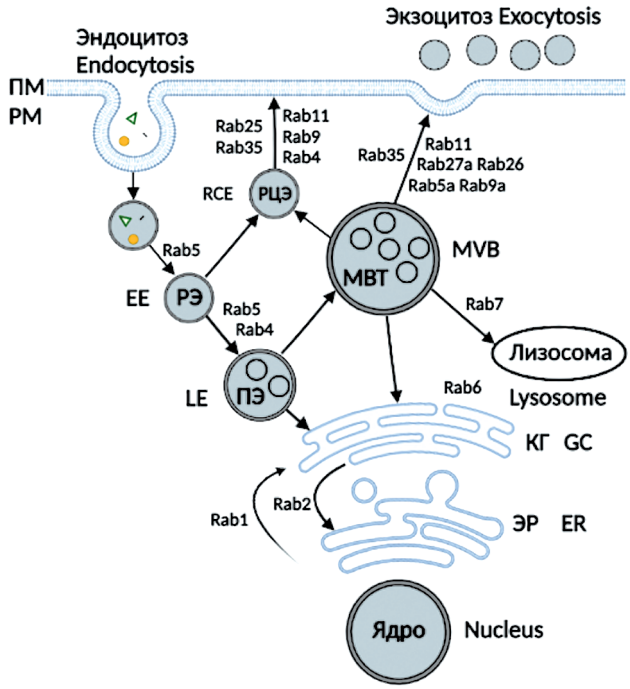

Fig. 2. Scheme of exosome biogenesis and participation of cellular proteins of Rab family.

Molecules and microparticles that penetrate into the cell by endocytosis are conventionally designated as a green triangle and a yellow circle. Rab5 participates in the fusion of endocytized vesicles to form an early endosome (EE); Rab5 and Rab4 – to the late endosome (LE); Rab1 regulates transport from the endoplasmic reticulum (ER) to the Golgi complex (GC). Rab2, on the contrary, participates in recycling and retrograde transport from GC back to ER. Rab6 regulates movements within GC. Rab7 regulates endosomal transport from LE and multivesicular bodies (MVB) to the lysosome. Rab4 and Rab11, as well as Rab 9 and Rab 25 regulate the processing of contents in recycling endosomes (RCE) and transport to the plasma membrane (PM). Rab27a and Rab35 are involved in the docking of MVB with the plasma membrane; Rab 11 and Rab35 are involved in the release of vesicles. Rab5a and Rab9a are also involved in the secretion of vesicles, enhancing the release of exosomes.

Рис. 2. Схема биогенеза экзосом и участие клеточных белков семейства Rab.

Молекулы и микрочастицы, проникающие в клетку путём эндоцитоза, условно обозначены как зелёный треугольник и жёлтый кружок. Rab5 участвует в слиянии эндоцитированных везикул с образованием ранней эндосомы (РЭ); Rab5 и Rab4 – транспорт к поздней эндосоме (ПЭ); Rab1 регулирует транспорт от эндоплазматического ретикулума (ЭР) к комплексу Гольджи (КГ). Rab2 участвует в рециркуляции и ретроградном транспорте от КГ обратно к ЭР. Rab6 регулирует перемещения внутри КГ. Rab7 регулирует эндосомальный транспорт от ПЭ и мультивезикулярных телец (МВТ) к лизосоме. Rab4 и Rab11, а также Rab 9 и Rab 25 регулируют переработку содержимого в рециклирующих эндосомах (РЦЭ) и транспортировку к плазматической мембране (ПМ). Rab27a и Rab35 участвуют в стыковке МВТ с плазматической мембраной; Rab 11 и Rab35 – в высвобождении везикул. В секреции везикул участвуют также Rab5a и Rab9a, усиливая выход экзосом.

The exosome biogenesis involves multiple cellular proteins [22]. Rab proteins belonging to the family of conserved GTPases of the Ras superfamily including more than 60 members have been studied quite thoroughly; one cell can contain more than 40 different Rab proteins [23]. The participation of some Rab proteins in various stages of exosome biogenesis is shown in Fig. 2. An important role in exosome biogenesis also belons to proteins of the endosomal sorting complex required for transport (ESCRT), including ESCRT-0, -I, -II, -III and accessory proteins, including ATPase Vps4. ESCRT-0, -I and -II participate in selective sorting of ubiquitinated molecules; the ESCRT-III and Vps4 complexes participate in membrane invagination and formation of ILVs comprising MVBs [24].

Role of exosomes in viral infections

Studies have demonstrated that exosomes can contain nucleic acids, proteins and even virions of enveloped viruses, which are transferred to recipient cells (Fig. 1). To create conditions favorable for replication and spread, viruses employ different strategies, affecting regulatory mechanisms of the cell and the body, including exosome biogenesis. The next sections of the review will focus on the main findings showing the participation of exosomes in four most common and socially significant viral infections caused by the human immunodeficiency virus type 1 (HIV-1), hepatitis B virus (HBV), hepatitis C virus (HCV) and SARS-CoV-2 coronavirus.

Exosomes and HIV-1 infection

After cells have been infected, HIV-1 recruits ESCRT-I, -II and -III complexes, which participate in processes of virus-cell interaction at different stages of the life cycle of the virus [25]. Proteins of the ESCRT-I complex – TSG101 and Alix are recruited to the HIV-1 assembly site. The same components are required to release HIV-1 from the infected cell [26, 27]. The Gag protein of HIV-1 is involved in the process of release from the cell, accumulating and anchoring at the plasma membrane. Studies of TSG101, the key component of ESCRT, using the CRISPR/Cas9 system in human cells infected with HIV-1 confirmed co-localization of the Tsg101 cellular protein with the HIV-1 Gag protein at the virus assembly sites. The inhibition of TSG101 synthesis or overexpression of the TSG101 gene severely impairs HIV-1 production by arresting the release of new virions from membranes of infected cells. Thus, the TSG101-HIV Gag interaction can be seen as a potential target for antiviral therapy [28–30]. The assembly of viral particles and secretion of exosomes are also regulated by proteins of the Rab family [31].

Exosomes have opposing effects in HIV pathogenesis. It has been found that exosomes released from T cells contain large amounts of CD4+ molecules that compete with host cells for binding to HIV-1 proteins, thus preventing the spread of the virus. Exosomes isolated from semen, breast milk and other body fluids can also have an antiviral effect, inhibiting HIV-1 replication [32]. On

the other hand, exosomes from HIV-1-infected cells can induce activation of CD4+ T lymphocytes and virus replication as well as in latently infected cells, thus leading to latent HIV reactivation from the viral reservoir in the body [33].

Exosomes and hepatitis B virus

Despite the availability of an effective preventive vaccine, hepatitis B remains a major public health problem, as no curative treatment of chronic hepatitis infection currently exists. This can partly be explained by the absence of complete understanding of HBV assembly and release pathways. It is believed that HBV enters the cell through endocytosis; Rab5 and Rab7 participate in HBV internalization and transportation from EEs to LEs [34]. Interestingly, at the late stages of HBV replication, the same Rab7 facilitates the transportation of viral particles to lysosomes for their further degradation [35]. In addition to Rab proteins, which act as molecular switches in vesicular transport in HBV infection, the virus uses ESCRT protein complexes [36, 37]. Note that intact HBV virions can be released as exosomes and transferred not only to susceptible, but also to insusceptible cells [38]. Thus, for its own replication and spread, HBV exploits the endocytic pathway involving Rab and ESCRT cellular systems.

Studies have demonstrated that exosomes can play a role in diagnosis and treatment of hepatitis B. Patients with active HBV replication (HBeAg-positive) had higher levels of some microRNAs (miRNAs) in plasma EVs compared to HBeAg-negative patients, and the levels of these miRNAs correlated with HBV DNA and hepatitis B surface antigen (HBsAg) levels [39]. The comparative study of exosomes in the blood of healthy volunteers, HBV carriers and patients with chronic hepatitis B led to the conclusion that exosomal miRNAs can be used as biomarkers for detection of different stages of HBV infection [40]. It has been observed that the CD63 tetraspanin associated with ILVs and exosomes colocalized with structural HBV proteins LHBs and hepatitis B core (HBc). Suppression of CD63 expression by RNA interference demonstrated that the CD63 tetraspanin plays an important role in HBV assembly and infectivity [41]. Other researchers isolated exosomes from the culture supernatant of HepAD38 hepatocytes with HBV replication (the cell line similar to HepG2.2.15) and used them for treatment of human peripheral blood mononuclear cells (PBMCs) [42]. Exosomes targeted monocytes, but not lymphocytes, causing upregulation of PD-L1 expression on monocytes and downregulation of expression of CD69, which is a marker of activated immune cells. Consequently, exosomes secreted by HepAD38 cells can induce immune suppression, depletion of T cells and potentially can contribute to progression of HBV infection. At the same time, reverse transcriptase inhibitors (entecavir, lamivudine and tenofovir) alter the contents of exosomes, leading to reduction of the immunoregulatory potential of the virus [42]. Further studies are required to provide a more detailed assessment of the impact of exosomes on hepatitis B pathogenesis.

Exosomes and hepatitis C virus

HCV causes acute infection that in most cases progresses into chronic hepatitis C (CHC), causing fibrosis, cirrhosis and hepatocellular carcinoma. Although more than 50 million people have CHC, there is no vaccine against hepatitis C, while the high price of direct-acting antivirals makes them unaffordable to many patients; they do not protect from re-infection and hepatocellular carcinoma recurrence after direct-acting antiviral therapy. Therefore, the development of new agents and, most importantly, the understanding of viral disease pathogenesis require more thorough knowledge of the virus life cycle and virus interaction with cellular processes. The studies of the HCV life cycle have demonstrated that the virus can be released from cells in the form of exosomes through the endosomal pathway [43, 44]. Two cell systems – ESCRT and Rab family proteins – are involved in endosomal trafficking [45–48]. The active participation of Rab family proteins suggests the prospect of using inhibitors that can suppress or block HCV infection. Such inhibitors have not been produced so far; however, strategies for the control of Rab GTPases are being looked for [49]. Envelope proteins and the HCV core protein were detected in ILVs of MVBs and were found to be also localized in exosomes, where the ESCRT-0 HRS (hepatocyte growth factor-regulated tyrosine kinase substrate) component is critical for release of HCV in exosomes [50, 51]. Interestingly, HRS is also required for secretion of exosomes from dendritic cells and regulation of antigen-presentation activity through exosomes [52]. The authors believe that deeper insights into the interaction between HCV and ESCRT cellular pathways can help select new targets for broad-spectrum antiviral therapy [53]. The fact that exosomes contain HCV structural and non-structural proteins as well as RNA makes it possible to see exosomes as a source of circulating biomarkers associated with pathological processes in hepatitis C. This is especially important in diagnosis of chronic hepatitis, considering that lack of accuracy of the current non-invasive methods is seen by international experts as a formidable obstacle for successful treatment of chronic diseases [54, 55].

The contents of exosomes can vary significantly depending on the nature of donor cells, physiological state, changes in the intracellular activity, and microenvironment. Thus, circulating exosomes derived solely from liver cells are of special interest. Compared to circulating proteins and RNA complexes, exosomes are characterized by high stability in body fluids, which is provided by lipid bilayer membranes. Note that changes in exosomes can be detected at earlier stages preceding apparent tissue damage or other clinical and histological features, as it was found using the mouse model of nonalcoholic steatohepatitis [56]. Diagnosis of hepatitis C involves serological and molecular methods, which are effectively used for assessment of the viral load and immune status of a patient. Development of

exosome-based diagnostic approaches can provide efficient non-invasive tools for more extensive and improved assessment of liver injury and for detection of early markers of the increased risk of hepatocellular carcinoma. Using exosomes as CHC biomarkers can be a challenging task, as it is technically difficult to isolate exosomes produced in liver cells from the large pool of circulating exosomes [57]. Overcoming these challenges will open up new possibilities in diagnosis of CHC, including application of liquid biopsy.

Experiments have shown that purified exosomes isolated from HCV-infected human hepatoma Huh7.5.1 cells contained full-length viral RNA, proteins and viral particles and were capable of transmitting productive HCV infection to naive hepatocytes [58]. In addition, it was found that antibodies from patients with hepatitis C only partially neutralized HCV infection transmitted through exosomes compared to the free virus. This suggests that HCV transmission by exosomes can be a potential immune evasion mechanism in hepatitis C [58]. The answer to the question whether HCV can spread through exosomes was looked for by analyzing if the virus could use exosomes for receptor-independent transmission of HCV to hepatocytes [59]. It was found that sera from all HCV-infected non-responder (interferon (IFN) α2b + ribavirin) patients and from some treatment-naïve patients had exosomes containing HCV negative sense RNA associated with replication of viral RNA. It was found that in exosomes HCV RNA was in complex with argonaute-2 (Ago2) protein, heat shock protein 90 (HSP90) and microRNA miR-122 **(Fig. 3)**. The researchers believe that their findings provide evidence for HCV transmission by circulating exosomes and highlight potential therapeutic strategies based on blocking exosomes from transmitting HCV infection. In the meantime, the discovery of the antisense strand may not be indicative of exosome-mediated transmission of the virus, as its transfer to cells will not provide virus genome translation and production of HCV proteins – components of the replication complex. Note that exosomes can also have the opposite effect. For example, exosomes containing HCV RNA could transport viral RNA to dendritic cells (pDCs); HCV RNA affected TLR7, activated pDC, promoted the production and secretion of IFN-α, thus causing inhibition of HCV replication and spread [60, 61].

Prospects of exosomes in hepatitis C treatment

The proteomic analysis identified around 250 proteins in EVs isolated from primary rat hepatocytes [62]. Around 70 proteins were identified in circulating EVs

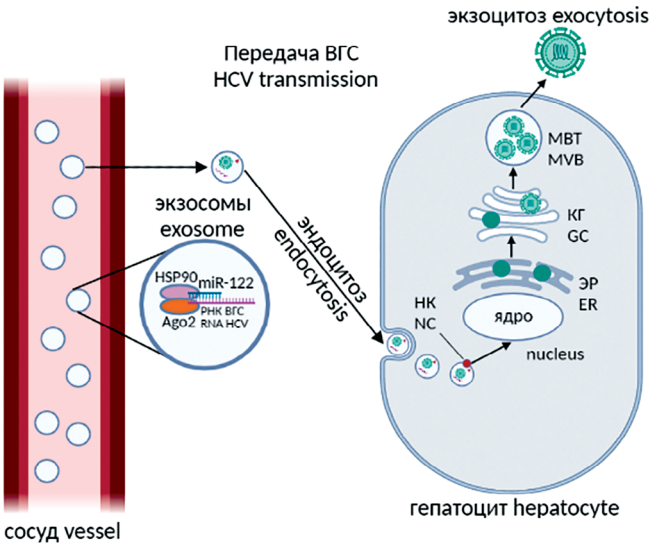

Fig. 3. Schematic representation of hepatitis C virus transmission through exosomes.

Exosomes containing HCV RNA in combination with microRNA (miR-122], heat shock protein HSP90 and argonaute-2 protein (Ago2) were found in the blood sera of patients with hepatitis C. Exosomes are able to penetrate uninfected cells, including hepatocytes, by endocytosis. In hepatocytes that have captured exosomes containing viral RNA, a productive HCV infection is observed, which can be transmitted through viral particles in the released exosomes. Thus, the spread of the virus is possible through exosomes, bypassing cellular receptors. NC – HCV nucleocapsid, ER – endoplasmic reticulum, GC – Golgi complex, MVB – multivesicular bodies, сосуд – vessel, экзосомы – exosomes, гепатоцит – hepatocyte, ядро – nucleus, эндоцитоз – endocytosis, экзоцитоз – exocytosis (according to T.N. Bukong, et al. [59]).

Рис. 3. Схематическое представление передачи вируса гепатита С через экзосомы.

В сыворотках крови пациентов с гепатитом С обнаружены экзосомы, содержащие РНК ВГС в комплексе с микроРНК (miR-122), белком теплового шока HSP90 и белком argonaute-2 (Ago2). Экзосомы способны проникать в незаражённые клетки, в том числе в гепатоциты, путём эндоцитоза. В гепатоцитах, захвативших экзосомы, содержащие вирусную РНК, наблюдается продуктивная ВГС-инфекция, которая может передаваться через вирусные частицы в высвобождающихся экзосомах. Таким образом, распространение вируса возможно через экзосомы, минуя клеточные рецепторы. НК – нуклеокапсид ВГС, ЭР – эндоплазматический ретикулум, КГ – комплекс Гольджи, МВТ – мультивезикулярные тельца (по T.N. Bukong и соавт. [59]).

from healthy people; most of them participate in transport of vesicles [63]. In addition to classical markers detected in most of the EVs from other types of cells, exosomes from hepatocytes had specific components. Moreover, almost all types of immune cells, including T and B lymphocytes, dendritic cells, macrophages, Kupffer cells, and neutrophils, produce exosomes. Therefore, attention should be given to the studies aimed at finding out the relationship between pathological changes in the liver and the contents of exosomes isolated from liver cells of patients with hepatitis. Studies have demonstrated that EVs/exosomes released from hepatocytes, unlike exosomes from other liver cells, have elevated levels of cytochrome P450 isoform 2E1 (CYP 2E1) and asialoglycoprotein receptor 1 [24]. It is assumed that the liver releases CYP-containing EVs to promote drug metabolism in other cells that absorb these vesicles. The publication offers the list of molecules found in EVs, which are associated with development of liver diseases [24]. Liver cells can be both donors and recipients of exosomes, demonstrating a complex interaction among different liver cells, involving proteins, mRNAs and microRNAs. Exosomes can be used as potential biomarkers in different liver diseases [64]. In hepatitis C, exosomes isolated from sera had increased levels of four miRs; in alcoholic hepatitis, hepatocyte-derived exosomes contain miRs that are associated with hyperinflammation; in hepatocellular carcinoma, exosomes were found to have CEACAM1/6 (carcinoembryonic antigen-related cell adhesion molecules) associated with tumor progression [64].

It has been found that EVs have multiple advantages over free proteins and nucleic acids, including the following:

1) nucleic acids do not replicate after administration;

2) substances contained in exosomes can have lower immunogenicity;

3) they possess an intrinsic ability to cross tissue and cellular barriers;

4) they are resistant to degradation by proteases in circulation and to freezing/thawing during long-term storage [65].

One of the therapeutic approaches aimed at inhibition of persistent HCV infection is offered in the following publication [66]. It has been found that compounds disrupting the endosomal pathway of exosome formation and the release of vesicles from cells can significantly inhibit replication of the virus in Huh7.5 cells infected with chimeric HCV containing the green fluorescent protein (GFP), while having no effect on the viability of the cells. Other approaches can be aimed at removal of circulating exosomes containing virions or HCV RNA as well as at prevention of their entry into target cells. “Harmful” exosomes can be removed from the circulation, for example, using methods similar to removal of circulating antibodies with extracorporeal dialysis. Other strategies for reduction or selection of specific types of exosomes have also been described [67, 68]. Most of the studies of the role of exosomes in treatment of hepatitis C have been performed using mesenchymal stem/stromal cells (MSCs). MSC-based cell therapy gains attention from many researchers; exogenous or activated endogenous MSCs are used in more than 1,200 clinical trials [69]. Clinical studies have demonstrated that MSC therapy alleviates liver damage, improves liver function, and promotes liver tissue regeneration. In acute or chronic liver failure, treatment with MSCs leads to higher survival rates, has good tolerance and safety rates [70]. In 2016, Qian et al. were the first to demonstrate the ability of exosomes produced by MSCs to inhibit HCV infection [71]. In the above studies, exosomes secreted from umbilical MSCs (uMSC-Exo) were non-toxic and inhibited replication of HCV *in vitro*. Specific miRs from the exosomes were actively involved in the process. In addition, exosomes from uMSC enhanced the effect of IFN-α and telaprevir used for CHC treatment.

A serious challenge in the treatment of all liver diseases, including hepatitis C, is posed by liver fibrosis that develops following chronic liver injury. The key role in this process is played by hepatic stellate cells (HSCs), which are activated to become myofibroblasts promoting deposition of extracellular matrix in the liver and, consequently, progression of fibrosis [72]. EVs and exosomes can play controversial roles in liver fibrosis. The authors [73] demonstrated that miR-19a from HCV-infected hepatocytes activated the STAT3-TGFβ pathway by activating HSCs. MiR-192 from HCV-infected cells was also able to activate HSCs through TGFβ upregulation in stellate cells [74]. Other studies demonstrated opposite effects. The exosomes derived from human liver stem cells inhibited profibrotic activity of stellate cells *in vivo* [75], and that effect was associated with delivery of antifibrotic miR-146a-5p through exosomes [76]. It has been reported that bone marrow MSC-derived exosomes can inhibit activation of HSCs *in vivo* and *in vitro* through the Wnt/β-catenin pathway [77]. It has been found that miR-486-5p contained in exosomes from human tonsil-derived MSCs binds to the 3′ untranslated region (UTR) of SMO mRNA and inhibits its expression, thus causing inactivation of HSCs [78]. Down-regulated miR-150-5p and elevated chemokine CXCL1 expression levels were detected in liver fibrosis. The transfer of miR-150-5p to HSCs through EVs derived from MSCs, inhibited activation of HSCs by inhibiting the CXCL1 expression [79]. Serum EVs from normal mice were administered to mice with experimentally induced liver fibrosis, resulting in reduced levels of hepatocyte death, inflammation, aspartate aminotransferase/alanine aminotransferase levels and pro-inflammatory cytokines in the liver and peripheral blood. Serum EVs from fibrotic mice did not have such effects [80]. It was found that activated HSCs were the primary targets for EVs. Levels of some miRs were higher in EVs from normal mice compared to EVs from fibrotic mice. Each miR was able to individually suppress fibrogenic gene expression in activated HSCs. Similar features were demonstrated by activated human HSCs: serum EVs from healthy people downregulated activation of HSCs and contained higher miR levels than EVs from patients with liver fibrosis.

The analysis of the role of EVs/exosomes in liver fibrosis suggests that:

1) HSCs and fibrosis development can be affected by EVs and exosomes from various sources – MSCs, hepatocytes, plasma, immune cells;

2) EVs and exosomes carry and transfer biologically active molecules, including proteins, mRNA, miR to activated HSCs;

3) EVs and exosomes that entered activated HSCs can inhibit molecular pathways, which involve LPS/TLR, STAT3/Bcl-2/Beclin-1, TGFβ/SMAD, Wnt/Beta-catenin etc.;

4) in liver fibrosis, the EV uptake by activated HSCs reduces HSC proliferation, decreases collagen maturation and pro-inflammatory cytokine levels, and upregulated autophagy.

Thus, myofibroblasts can revert to a quiescent state [81]. It has been assumed that serum EVs and exosomes from healthy individuals are inherently anti-fibrogenic and anti-fibrotic, and contain miRs that have therapeutic actions in activated stellate cells or injured hepatocytes [80]. It has been noted that specific alterations in the miR profile in EVs can be seen as potential diagnostic biomarkers to differentiate between different types and stages of progression of chronic hepatitis [82]. At the same time, the findings [83] have shown that EVs/exosomes containing certain miRs can also be seen as potential therapeutic agents. Interesting observations were made in studies of natural killer cells (NK cells) that participate in HSC activation [84] and can affect functions of target cells through exosome secretion [85]. It has been found that NK cells (NK-92MI cell line) excrete exosomes (NK-Exos). After their purification, NK-Exos were added to the culture containing activated human HSCs of the LX-2 cell line as well as injected into mice with CCl4-induced fibrosis **(Fig. 4)**. The NK-Exo treatment significantly inhibited HSC proliferation and activation *in vitro*. Moreover, NK-Exos alleviated liver fibrosis in mice [86]. Presumably, the effect was associated with miR-223 that was highly expressed in exosomes released from NK-Exos. Indeed, the inhibition of miR-223 expression in NK-Exo significantly abrogated the inhibitory effect of NK-Exo on HSC activation. Using the TargetScan software, ATG7, one of the autophagy markers, was identified as a putative target of miR-223 [87]. The assay confirmed that ATG7 was a direct target of miR-223. Since autophagy can be involved in HSC activation, it can be concluded that exosomes from NK cells inhibit HSC activation by transferring miR-223 that inhibited autophagy via targeting ATG7 [88]. These findings correlate with the studies demonstrating that inhibition of autophagy inhibits development of liver fibrosis [89, 90]. The obtained results highlight prospects for further research focused on development of an exosome-based delivery system for treatment of liver diseases, including chronic viral hepatitis. At the same time, it is still unclear how recipient cells recognize EVs and how EVs interact with target cells *in vivo*. Some authors mention different molecules on the surface of recipient cells that EVs can bind to [91]. The *in vivo* model was developed to study EV functions by expressing CD63-pHluorin in zebrafish embryos. Having detected exosomes in the circulation, the

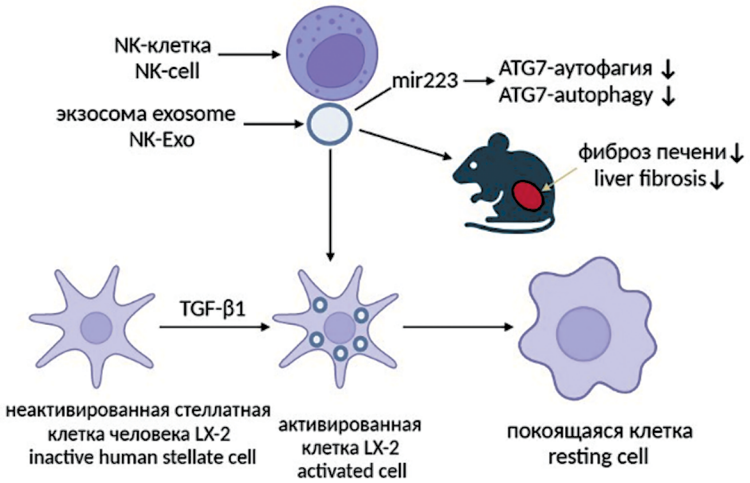

Fig. 4. Exosomes from natural killer (NK) cells reduce the level of experimental liver fibrosis.

Inactive human hepatic stellate cells of the LX-2 line were activated with TGF-β1 and then treated with exosomes isolated from NK cells – NK-Exo exosomes. As a result, human liver stellate cells activity was suppressed. The injection of NK-Exo into mice with experimentally induced fibrosis led to a decrease in the level of fibrosis. The antifibrotic effect of NK-Exo was associated with a high level of miR-223 expression directed at the autophagy protein ATG7 and suppression of its function. The blockade of autophagy caused a decrease in the level of liver fibrosis (according to L. Wang, et al. [86, 88]).

Рис. 4. Экзосомы из клеток натуральных киллеров (NK) снижают уровень экспериментального фиброза печени.

Неактивные стеллатные клетки печени человека линии LX-2 активировали с помощью TGF-β1 и затем обрабатывали экзосомами, выделенными из клеток NK – NK-Exo. В результате активность стеллатных клеток была подавлена. Введение NK-Exo мышам с экспериментально вызванным фиброзом приводило к снижению уровня фиброза. Антифиброзное действие NK-Exo ассоциировалось с высоким уровнем экспрессии микроРНК miR-223, направленной на белок аутофагии ATG7 и подавление его функции. Блокада аутофагии вызывала снижение уровня фиброза печени (по L. Wang и соавт. [86, 88]).

authors concluded that exosomes were endocytosed by patrolling macrophages and endothelial cells in the caudal vein plexus [92]. The role of macrophages in exosome recognition was also pointed out in the mice-involving study [93], that demonstrated the importance of phosphatidylserine-derived negative charges in exosome membranes in recognition of intravenously injected exosomes by macrophages.

Authors studying exosomes, EVs, and EVs isolated from MSCs (MSC-EVs) point out growing interest in the role of MSC-EVs in liver diseases [94]. MSC-EVs are more convenient and less immunogenic than MSCs; they do not engraft, have intrinsic liver tropism, do not cause aberrant stem cell differentiation, have low immunogenicity and no risk of tumorigenicity. MSC-EV-based cell-free therapy as well as EVs modified with antiviral molecules open up new avenues for treatment of liver diseases.

Exosomes in hepatitis C prevention

Exosomes can be seen as potential vaccines due to their properties. However, there have been only a few studies addressing this prospect. It has been noted that exosomes can improve distribution of antigens due to their ability to circulate in body fluids and reach distal organs [95]. It has been found that HCV RNA-containing exosomes from HCV infected cells can induce IFN-α production in uninfected plasmacytoid dendritic cells [60]. Exosomal export of viral RNA can serve as a viral strategy to evade pathogen detection and as host strategies to induce an immune response. It has been demonstrated that exosomes can enter hepatocytes infected with hepatitis B virus and transmit IFN-α, activating the respective antiviral cascade [96]. These and other findings suggest that exosomes can be not only antigen transmitters, but also inducers of an immune response.

Exosomes and coronavirus infection

Coronaviruses can enter cells by direct fusion of the virus membrane with the plasma membrane of cells or by endocytosis. There are findings suggesting that exosomes may participate in the entry of coronavirus into target cells. It has been found that protein complexes with cellular receptors of coronaviruses and TMPRSS2 protease contain CD9 tetraspanins, which, along with CD81 and CD63, are an integral part of exosome membranes [97]. The complexes play an important role in the fast and efficient entry of coronaviruses. In the absence of CD9, viruses use cathepsins to enter cells, though much later and less efficiently [98]. Exosomes released from cells infected with coronavirus can facilitate virus entry into uninfected cells by transferring CD9 molecules.

Exosomes in the pathogenesis of COVID-19

Exosomes from patients with COVID-19 can contain viral RNA, proteins, and even SARS-CoV-2 virions [99, 100]. One of the studies in this field [101] was performed using exosomes isolated from plasma from 20 patients with COVID-19 and 8 healthy volunteers; the proteomic analysis was used to analyze exosomes. 163 proteins out of 1637 identified proteins were shortlisted as having more counts compared with exosomes from healthy individuals. Two proteins – tenascin-C (TNC) and fibrinogen-β (FGB) demonstrated especially significant changes in their contents – more than 200-fold and 700-fold, respectively. Immortalized hepatocytes IHHs and Huh7s were used to identify the possibility of exome content transfer to other cells. Cells of both cell lines had TNC and FGB, and higher counts of these proteins were detected in hepatocytes treated with exosomes from patients with COVID-19. The possibility of association between exosomes and inflammation was analyzed, considering that TNC is an immunomodulator that can induce chronic inflammation [102], and elevated levels of FGB in blood are associated with vascular disorders observed in patients with COVID-19. The analysis focused on expression of tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6) and chemokine ligand 5 (CCL5) in hepatocytes exposed to exosomes from patients with COVID-19 and from healthy individuals. It was found out that the expression of cytokines and chemokine in hepatocytes was significantly increased only after treatment with exosomes from patients with COVID-19 and was associated with NF-kB activation in hepatocytes **(Fig. 5** *a***)**. These findings imply that exosomes from plasma from patients with COVID-19 can potentially induce production of pro-inflammatory cytokines and promote clinical manifestations of the SARS-CoV-2-associated disease not only in lungs, but also in hepatocytes – cells of a distant organ. This conclusion is supported by observations regarding extrapulmonary manifestations of COVID-19 [103].

The role of EVs/exosomes in SARS-CoV-2 infection was studied using tests with A549 lung epithelial cells [104]. It was reported that after transduction with lentivirus encoding two nonstructural (Nsp1 and Nsp12) and two structural (envelope E and nucleocapsid N) SARS-CoV-2 proteins, EVs isolated from A549 cells were found to contain viral RNA **(Fig. 5** *b***)**. EVs were used for treatment of cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs). The qRT-PCR test detected mRNAs of all four viral genes in cardiomyocytes. Moreover, the expression of pro-inflammatory genes IL-1β, IL-6 and MCP1 was significantly increased in cardiomyocytes containing Nsp1. The findings indicate that the cells that do not express ACE2 SARS-CoV-2 receptors can receive viral genetic information by taking up EVs/exosomes, and the expression of viral genes can contribute to inflammation, being typical of the pathogenesis of COVID-19. At the same time, EVs/exosomes can deliver ACE2 to cells with absent or poor expression of SARS-CoV-2 receptors by transporting the protein from other cells [105]. Exosomes containing ACE2 (ACE2+) were identified in plasma from patients with COVID-19 and it was found [106] that ACE2+ exosomes competed with cellular ACE2 for SARS-CoV-2 neutralization by inhibiting binding of the viral S protein to ACE2+ cells in a dose dependent manner **(Fig. 5** *c***)**. Exosomes containing ACE2 were 120-135 times more efficient blocking the receptor-binding domain (RBD) of S protein compared to vesicle-free recombinant human ACE2 (rhACE2) [107].

Furthermore, ACE2+ exosomes were 60–80 times more efficient in protecting transgenic mice expressing human *ace2* gene from lung injury and death after intranasal inoculation of SARS-CoV-2. ACE2+ exosomes inhibited infection with SARS-CoV-2 α, β and δ variants having mutations in RBD, demonstrating the same or higher effectiveness than wild-type strain. These findings showed that exosomes containing ACE2 can serve as a basis for development of broad-spectrum therapeutic agents against emerging and re-emerging coronaviruses using the ACE2 receptor [108].

Interesting results were obtained in studies involving patients with different severity of COVID-19 [109]. They presented quantitative data of studying 1002 metabolites in plasma, showing a significant increase in levels of GM3 gangliosides, which correlated with the decrease in counts of circulating CD4+ T cells in patients with COVID-19 and the progressive increase in indices of systemic inflammation, including C-reactive protein, IL-6, erythrocyte sedimentation rate, serum ferritin and procalcitonin as the disease severity increased. The comparative analysis revealed a strong correlation between the disease severity and the detection of ganglioside (GM3)-enriched exosomes in sera **(Fig. 5** *d***)**. It was found that GD3s expressed on the surface of exosomes in the tumor microenvironment inhibited functions of T cells, contributing to immunosuppression [110]. Gangliosides are found in all vertebrate cells; they are expressed on the outer surface of plasma membranes [111], bind specifically to regulatory proteins and other molecules, modulate the activity of membrane proteins and act as receptors in intercellular interactions; they are targets for pathogens, including SARS-CoV-2 [112]. Thus, ganglioside-containing exosomes detected in the circulation can worsen COVID-19 by disrupting cellular regulatory pathways and the immune response [113].

Formation of blood clots is one of the symptoms of COVID-19. The clinical study showed that patients with COVID-19 had circulating EVs containing active CD142 molecules closely associated with increased procoagulant activity **(Fig. 5** *e***)**. The release of CD142-containing EVs from endothelial cells is associated with pro-inflammatory activity [114]. In COVID-19, the vascular system is also affected by CD142-loaded EVs from platelets. Counts of such vesicles significantly increase in the circulation of patients with COVID-19. The direct relationship between circulating platelet-derived EVs and the disease severity has been found, serving as a ground for offering these vesicles as biomarkers to be used in prediction of outcomes in patients with COVID-19 [99, 115–117].

Therefore, we can conclude that the participation of exosomes isolated from cells of patients infected with SARS-CoV-2 in the pathogenesis of COVID-19 depends on:

a) levels of viral RNA, proteins and virions;

b) transport of viral components from the entry points (respiratory epithelium) to other organs;

c) the ability to alter the expression of cellular genes involved in the pathogenesis COVID-19;

d) increased vascular dysfunction and cytokine storm.

Exosomes in treatment and prevention of COVID-19

The wide range of currently available vaccines decreases the burden of SARS-CoV-2 infection; however, a significant number of patients who develop pneumonia and other serious diseases still need treatment. Among numerous agents and strategies offered for therapy by different researchers, dozens of publications and clinical trials are focused on studying of the possible use of MSCs of various origins [69]. It has been found that MSCs inhibit inflammation, improve lung functions, do not cause side effects, and significantly reduce mortality among patients with COVID-19 [118]. The authors have concluded that MSCs are a safe and effective tool for treatment of COVID-19. Studies are not over, as none of the tested products has been approved for use in treatment of COVID-19.

Lately, the attention of researchers has been attracted by cell-free therapy that has a number of advantages over the MSC-therapy [119]. It employs MSCs secretome (a set of factors and biomolecules secreted by cells) and EVs/exosomes released from MSCs. The analysis of the effect of the MSCs secretome on injured rat lungs demonstrated the improvement in the lung architecture, a decrease in α-SMA and reduction in collagen levels. This led to the assumption that the MSCs secretome can launch a new therapeutic approach to treatment of such severe complications of COVID-19 as pulmonary fibrosis [120].

The findings of the researchers who analyzed the role of exosomes in severe lung damage paved the way for using exosomes for treatment of COVID-19. The results obtained during some studies showed that EVs/exosomes derived from human bone marrow MSCs have a beneficial therapeutic effect in acute lung injury and acute respiratory distress syndrome in laboratory animals [121]. Thanks to the presence of biologically active molecules in exosomes, these vesicles can activate the regeneration of injured tissues, suppress the production of inflammatory cytokines, and modulate functions of immune cells [122].

One of the advantages of exosomes is associated with their ability to penetrate various organs and tissues, thus demonstrating their capacity as therapeutic agents for aerosol inhalation (nebulizer therapy) [123, 124]. It has been demonstrated that inhalation of secretome and exosomes obtained by culturing cells from mouse lungs containing epithelial, progenitor cells, and MSCs can facilitate lung recovery in fibrosis. The collagen accumulation and the myofibroblast proliferation start decreasing; the normal alveolar structure of the lungs is reestablished [125]. The inhalation method of administration is less painful; it has a faster effect, and at lower doses, demonstrates the same effect as oral or injection therapy [121].

It has been noted that EVs/exosomes can have both a positive and a negative role in coronavirus infection. On the one hand, EVs can inhibit and prevent infection, as it was shown by the example of EVs containing ACE2 or ACE2 + TMPRSS2 [126, 127]. On the other hand, EVs can promote viral infection by trapping and spreading the virus or viral components and protecting them from the

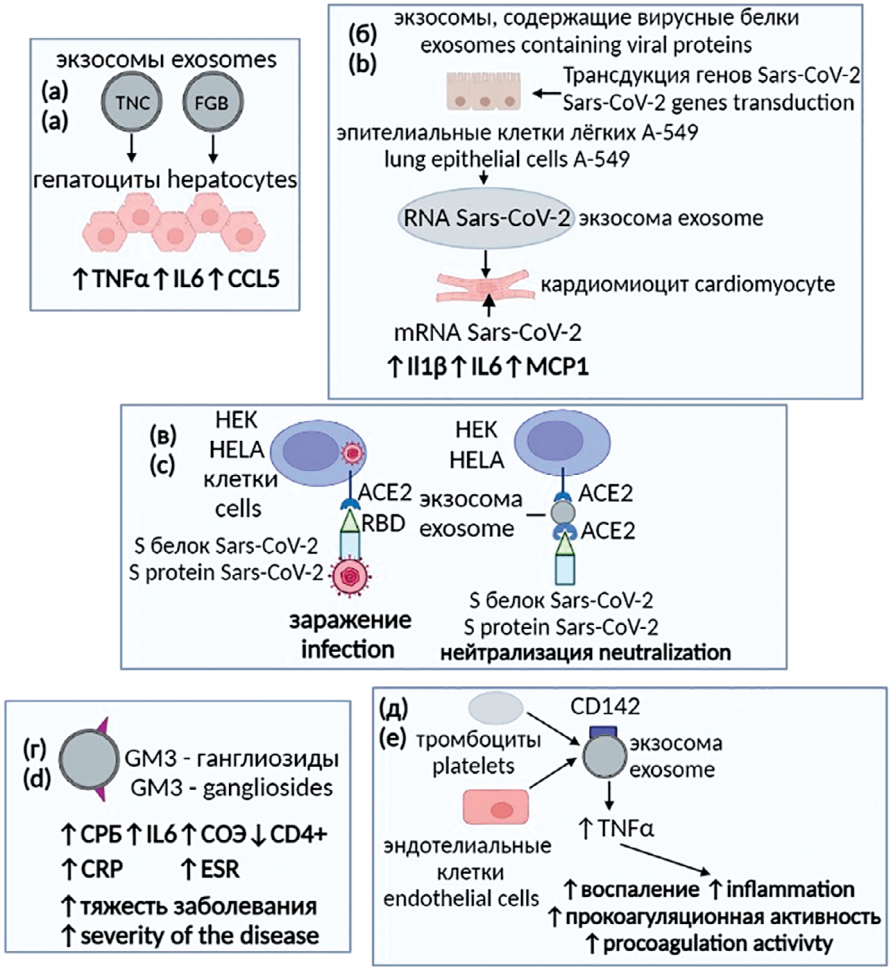

Fig. 5. Exosomes in the pathogenesis of COVID-19:

a – exosomes secreted by cells infected with SARS-CoV-2 are enriched with tenascin-C (TNC) and fibrinogen-β (FGB) and, penetrating into hepatocytes, initiate the production of TNF-α, IL-6 and CCL5 in hepatocytes (according to S. Sur, et al. [101]);

b – exosomes containing SARS-CoV-2 genes were introduced into the culture of cardiomyocytes and the expression of viral genes and a significant increase in the expression of proinflammatory cytokines and chemokines were observed in cardiomyocytes (according to Y. Kwon, et al. [104];

c – cell receptor ACE2 interacts with the receptor-binding domain (RBD) of SARS-CoV-2 S protein, causing cell infection (left); exosomes containing ACE2 were found in the blood of patients with COVID-19. They competed with the ACE2 cell receptor, blocking the binding of S protein to cells and neutralizing the infectious activity of the virus (right) (according to L. El-Shennawy, et al. [107]);

d – in the blood sera of patients with COVID-19, an increase in the number of exosomes carrying GM3 gangliosides on the surface was found. The presence of GM3-exosomes was accompanied by an increase in C-reactive protein (CRP), interleukin 6 (IL-6) and ESR rate, a decrease in the number of $CD4^+$ cells, and also correlated with the severity of the disease (according to J.W. Song et al. [109]);

e – exosomes released from endothelial cells and platelets into circulation of patients with COVID-19 contain active CD142 molecules and have increased pro-inflammatory and procoagulant activity, directly related to the severity of the disease (according to W. Holnthoner, et al. [114] and C. Balbi, et al. [115]).

Рис. 5. Участие экзосом в патогенезе COVID-19:

а – экзосомы, секретируемые клетками, инфицированными SARS-CoV-2, обогащены тенасцином-С (TNC) и фибриногеном-β (FGB) и, проникая в гепатоциты, инициируют продукцию TNF-α, IL-6 и CCL5 в гепатоцитах (по S. Sur и соавт. [101]);

б – экзосомы, содержавшие гены SARS-CoV-2, вносили в культуру кардиомиоцитов и наблюдали в них экспрессию вирусных генов и значительное увеличение экспрессии генов провоспалительных цитокинов и хемокинов (по Y. Kwon и соавт. [104]);

в – клеточный рецептор ACE2, взаимодействует с рецептор-связывающим доменом (RBD) S-белка SARS-CoV-2, что вызывает заражение клеток (слева); в крови пациентов с COVID-19 обнаружили экзосомы, содержащие ACE2, и показали, что они конкурируют с клеточным рецептором ACE2, блокируя связывание S-белка с ACE2 клеток и нейтрализуя инфекционную активность вируса (справа) (по L. El-Shennawy и соавт. [107]);

г – в сыворотках крови пациентов с COVID-19 обнаружили увеличение количества экзосом, несущих на поверхности ганглиозиды GM3, присутствие которых сопровождалось увеличением С-реактивного белка (СРБ), интерлейкина 6 (IL-6) и скорости оседания эритроцитов (СОЭ), снижением количества $CD4^+$-клеток, а также коррелировало со степенью тяжести заболевания (по J.W. Song и соавт. [109]);

д – экзосомы, высвобождающиеся из эндотелиальных клеток и тромбоцитов в циркуляцию у пациентов с COVID-19, содержат активные молекулы CD142 и обладают повышенной провоспалительной и прокоагулянтной активностью, прямо связаны с тяжестью заболевания (по W. Holnthoner и соавт. [114] и C. Balbi и соавт. [115]).

immune system. Moreover, it has been assumed that the peripheral blood of patients with SARS-CoV-2 contains antagonistic exosomes, which can reduce the virus-neutralizing ability of plasma of convalescents and may act as a competing inhibitor of neutralizing antibodies [128].

Exosomes are used in clinical studies focusing on treatment of COVID-19 [129]. The potential of exosomes is assessed in three aspects:

1) as vesicles secreted by MSCs from various sources;

2) as vesicles containing specific microRNAs and mRNAs;

3) as vesicles delivering drugs for treatment of COVID-19.

As commercial exosome products are coming on stage, they are also included in studies of treatment effectiveness. For example, studies were performed addressing safety and efficacy of application of exosomes (ExoFlo) derived from allogeneic bone marrow MSCs in the treatment of 24 patients with severe COVID-19, moderate and severe acute respiratory distress syndrome [130]. The intravenous administration of ExoFlo demonstrated the safety of the product, its ability to restore oxygenation, to inhibit the cytokine storm and to re-establish the immunity. The authors have concluded that ExoFlo is a highly promising therapeutic product candidate for severe COVID-19. Summing up the data on the effect of exosomes on various cells in lung tissue infected with SARS-CoV-2, the authors concluded [129] that exosomes can: a) interact both with the SARS-CoV-2 S protein and with the ACE2 cell receptor, competitively inhibiting the virus entry; b) decrease levels of pro-inflammatory cytokines in vessel and alveolar cells; improve functions of macrophages, interferons, and B cells, modulating immune responses. These findings provide a clear proof that exosomes are promising candidates for development of vaccines.

The S protein of SARS-CoV-1 that caused the SARS outbreak in 2002-2003 came to the fore when the possibility of using exosomes for development of vaccines against coronaviruses was first considered [131]. The exosome-based products containing the S protein were administered to mice and demonstrated that two doses of injections of exosomes without adjuvants were sufficient for inducing neutralizing antibodies against the coronavirus. The highest effect was achieved when the immunization with exosomes was followed by the immunization with the adenoviral vector expressing S protein. The neutralizing activity of antibodies in sera from the immunized mice and their activity in sera from convalescent patients with pneumonia caused by SARS-CoV-1 were compared. It was found that after the first immunization with the exosome-based vaccine and the booster immunization with the adenoviral vector, the neutralizing activity of antibodies exceeded the activity observed in the sera from the convalescent patients. The highly effective induction of a humoral response to the exosome vaccine containing the SARS-CoV-2 S protein demonstrated the feasibility of the further studies of protective properties of novel exosome vaccines capable of preventing coronavirus infections.

Most of the vaccines used against SARS-CoV-2 are intended for intramuscular injection [132]. In 2022, the development and preclinical trial of the inhalable vaccine against COVID-19 were announced; after lyophilization, the vaccine remains stable at room temperature for more than three months [133]. The vaccine consists of a SARS-CoV-2 RBD conjugated to human lung-derived exosomes **(Fig. 6)**. This design of the vaccine (exosomes containing RBD on the membrane) increases the RBD retention up to 21 days both in the respiratory mucosa and the lung parenchyma. The inhalation of the vaccine by mice produced specific IgG antibodies against RBD in blood and IgA antibodies in mucosa. The induction of $CD4^+$ and $CD8^+$ T cells expressing pro-inflammatory cytokines was observed in the lungs of the animals and their clearance from the SARS-CoV-2 pseudovirus after the infection. In hamsters, two doses of the vaccine alleviated the severe pneumonia and reduced the inflammatory infiltrates after the infection with live SARS-CoV-2. Exosomes containing a recombinant SARS-CoV-2 RBD (rRBD) on their membrane should be tested further as a candidate inhalable vaccine against COVID-19. The advantages of the inhalable vaccine can include the natural origin of nanoparticles – exosomes carrying viral antigen; fast and direct delivery of exosomes to the respiratory mucosa; absence of storage and shipping temperature limitations, and non-invasive method of application.

Conclusion

Assessing the role of exosomes in viral infections, their dual role should be pointed out. The ability to take up and transport viral RNA and DNA genomes, proteins and viral particles to uninfected cells can promote genetic cooperation between viral quasispecies, improve their fitness and extend their length of stay in the body. EVs/exosomes were called Trojan horses in viral infection due to their properties [134, 135]. Indeed, viruses can employ exosomes to propagate and enhance the infection as well as to be protected from the host immune response. In the meantime, there are findings evidencing the ability of exosomes to counteract viral infections. For example, it has been found that exosomes contain numerous antiviral factors that inhibit HIV-1 replication [136] by affecting the viral *Tat* gene and its complexes with cell genes [137, 138]. The positive role of exosomes produced by MSCs has been observed in clinical trials addressing acute respiratory infections [139, 140]. Exosomes isolated from culture of primary human trophoblasts provided resistance of recipient cells to a number of known viruses, including vaccinia virus, herpes simplex virus type 1 and cytomegalovirus.

Thus, the processes involving exosomes can have both a positive and a negative role in the pathogenesis of viral infections, either contributing to infection or inhibiting its development [141]. One of the important approaches to application of exosomes focuses on exosome-specific markers of viruses and viral infections, thus offering prospects for using exosomes as liquid biopsy and non-invasive diagnostics. With their natural origin from human

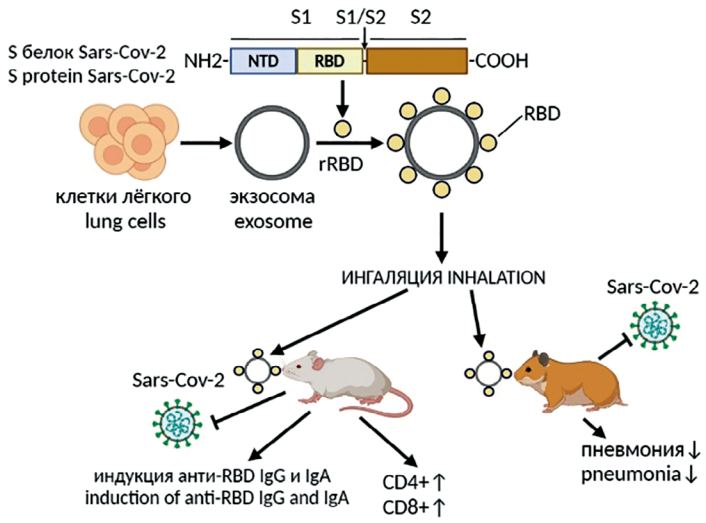

Fig. 6. Schematic representation of the preparation and effects of the inhaled vaccine against SARS-CoV-2.

S protein SARS-CoV-2 contains a receptor binding domain (RBD). Recombinant protein RBD (rRBD) has been prepared. Human lung cells were obtained by minimal invasive biopsy and three-dimensional cultivation, from which exosomes were isolated. The exosomes were conjugated with rRBD, which was localized on the membrane of the exosome. Exosomes were injected into mice and hamsters by inhalation. In mice, the exosomal vaccine induced the induction of IgG and mucosal IgA anti–RBD antibodies, an increase in the number of $CD4^+$ and $CD8^+$ cells, and virus clearance after infection with SARS-CoV-2. In hamsters, the vaccine weakened severe pneumonia caused by coronavirus (Adapted with modification from Z. Wang, et al. [186]).

Рис. 6. Схематическое изображение получения и действия ингаляционной вакцины против SARS-CoV-2.

S-белок SARS-CoV-2 содержит домен, связывающий рецептор (RBD). Получен рекомбинантный белок RBD (rRBD). Методом малоинвазивной биопсии и трехмерного культивирования получали клетки лёгкого человека, из которых выделяли экзосомы. Экзосомы конъюгировали с rRBD SARS-CoV-2, который локализовался на мембране экзосомы. Путем ингаляции экзосомы вводили мышам и хомякам. У мышей экзосомная вакцина вызывала индукцию анти-RBD антител классов IgG и мукозных – IgA, увеличение количества клеток $CD4^+$ и $CD8^+$ и клиренс вируса после заражения SARS-CoV-2. У хомяков вакцина ослабляла тяжелую пневмонию, вызванную коронавирусом (по Z. Wang и и соавт. [186], модифицировано).

cells and high biocompatibility, exosomes can be used for drug delivery. Genetically engineered exosomes can serve as a basis for developing therapeutical agents and antiviral vaccines. Obviously, the positive and negative role of exosomes in the pathogenesis of viral infections has not been sufficiently studied and needs to be explored further. For example, the use of exosomes for therapeutic and preventive purposes can pose a threat due to the risk of developing autoimmune responses, as exosomes have human components (including proteins) in their structure. The improving methods of isolation, purification, and standardization of vesicles as well as further multifaceted studies of their properties open prospects for using exosomes in the fight against viral infections.

ЛИТЕРАТУРА/REFERENCES

1. Harding C., Heuser J., Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J. Cell Biol*. 1983; 97(2): 329–39. https://doi.org/10.1083/jcb.97.2.329
2. Pan B.T., Johnstone R.M. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell*. 1983; 33(3): 967–78. https://doi.org/10.1016/0092-8674(83)90040-5
3. Harding C.V., Heuser J.E., Stahl P.D. Exosomes: looking back three decades and into the future. *J. Cell Biol*. 2013; 200(4): 367–71. https://doi.org/10.1083/jcb.201212113
4. Johnstone R.M., Adam M., Hammond J.R., Orr L., Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J. Biol. Chem*. 1987; 262(19): 9412–20.
5. Xie S., Zhang Q., Jiang L. Current knowledge on exosome biogenesis, cargo-sorting mechanism and therapeutic implications. *Membranes (Basel)*. 2022; 12(5): 498. https://doi.org/10.3390/membranes12050498
6. Kowal J., Arras G., Colombo M., Jouve M., Morath J.P., Primdal-Bengtson B., et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc. Natl Acad. Sci. USA*. 2016; 113(8): E968–77. https://doi.org/10.1073/pnas.1521230113
7. Wang S., Zhang K., Tan S., Xin J., Yuan Q., Xu H., et al. Circular RNAs in body fluids as cancer biomarkers: the new frontier of liquid biopsies. *Mol. Cancer*. 2021; 20(1): 13. https://doi.org/10.1186/s12943-020-01298-z
8. Kalluri R., LeBleu V.S. The biology, function, and biomedical applications of exosomes. *Science*. 2020; 367(6478): eaau6977. https://doi.org/10.1126/science.aau6977
9. Zeng Y., Qiu Y., Jiang W., Shen J., Yao X., He X., et al. Biological features of extracellular vesicles and challenges. *Front. Cell Dev. Biol*. 2022; 10: 816698. https://doi.org/10.3389/fcell.2022.816698
10. Todd K.V., Tripp R.A. Exosome-mediated human norovirus infection. *PLoS One*. 2020; 15(8): e0237044. https://doi.org/10.1371/journal.pone.0237044
11. Kowalczyk A., Wrzecińska M., Czerniawska-Piątkowska E., Kupczyński R. Exosomes – spectacular role in reproduction. *Biomed. Pharmacother*. 2022; 148: 112752. https://doi.org/10.1016/j.biopha.2022.112752
12. Lee I., Choi Y., Shin D.U., Kwon M., Kim S., Jung H., et al. Small extracellular vesicles as a new class of medicines. *Pharmaceutics*. 2023; 15(2): 325. https://doi.org/10.3390/pharmaceutics15020325
13. Picca A., Guerra F., Calvani R., Coelho-Junior H.J., Bucci C., Marzetti E. Circulating extracellular vesicles: friends and foes in neurodegeneration. *Neural. Regen. Res*. 2022; 17(3): 534–42. https://doi.org/10.4103/1673-5374.320972

14. Liu F., Vermesh O., Mani V., Ge T.J., Madsen S.J., Sabour A., et al. The exosome total isolation chip. *ACS Nano*. 2017; 11(11): 10712–23. https://doi.org/10.1021/acsnano.7b04878
15. Zhang H., Lyden D. Asymmetric-flow field-flow fractionation technology for exomere and small extracellular vesicle separation and characterization. *Nat. Protoc*. 2019; 14(4): 1027–53. https://doi.org/10.1038/s41596-019-0126-x
16. Kang Y.T., Kim Y.J., Bu J., Cho Y.H., Han S.W., Moon B.I. High-purity capture and release of circulating exosomes using an exosome-specific dual-patterned immunofiltration (ExoDIF) device. *Nanoscale*. 2017; 9(36): 13495–505. https://doi.org/10.1039/c7nr04557c
17. Pathan M., Fonseka P., Chitti S.V., Kang T., Sanwlani R., Van Deun J., et al. Vesiclepedia 2019: a compendium of RNA, proteins, lipids and metabolites in extracellular vesicles. *Nucleic. Acids. Res*. 2019; 47(D1): D516–9. https://doi.org/10.1093/nar/gky1029
18. Sidhom K., Obi P.O., Saleem A. A review of exosomal isolation methods: is size exclusion chromatography the best option? *Int. J. Mol. Sci*. 2020; 21(18): 6466. https://doi.org/10.3390/ijms21186466
19. van der Pol E., Sturk A., van Leeuwen T., Nieuwland R., Coumans F. Standardization of extracellular vesicle measurements by flow cytometry through vesicle diameter approximation. *J. Thromb. Haemost*. 2018; 16(6): 1236–45. https://doi.org/10.1111/jth.14009
20. Théry C., Witwer K.W., Aikawa E., Alcaraz M.J., Anderson J.D., Andriantsitohaina R., et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicles*. 2018; 7(1): 1535750. https://doi.org/10.1080/20013078.2018
21. Mulligan R.J., Yap C.C., Winckler B. Endosomal transport to lysosomes and the trans-Golgi network in neurons and other cells: visualizing maturational flux. *Methods Mol. Biol*. 2023; 2557: 595–618. https://doi.org/10.1007/978-1-0716-2639-9_36
22. Krylova S.V., Feng D. The machinery of exosomes: biogenesis, release, and uptake. *Int. J. Mol. Sci*. 2023; 24(2): 1337. https://doi.org/10.3390/ijms24021337
23. Homma Y., Hiragi S., Fukuda M. Rab family of small GTPases: an updated view on their regulation and functions. *FEBS J*. 2021; 288(1): 36–55. https://doi.org/10.1111/febs.15453
24. Liu G., Yin X.M. The role of extracellular vesicles in liver pathogenesis. *Am. J. Pathol*. 2022; 192(10): 1358–67. https://doi.org/10.1016/j.ajpath.2022.06.007
25. Scourfield E.J., Martin-Serrano J. Growing functions of the ESCRT machinery in cell biology and viral replication. *Biochem. Soc. Trans*. 2017; 45(3): 613–34. https://doi.org/10.1042/BST20160479
26. Lin C.Y., Urbina A.N., Wang W.H., Thitithanyanont A., Wang S.F. Virus hijacks host proteins and machinery for assembly and budding, with HIV-1 as an example. *Viruses*. 2022; 14(7): 1528. https://doi.org/10.3390/v14071528
27. Johnson D.S., Bleck M., Simon S.M. Timing of ESCRT-III protein recruitment and membrane scission during HIV-1 assembly. *Elife*. 2018; 7: e36221. https://doi.org/10.7554/eLife.36221
28. Hoffman H.K., Fernandez M.V., Groves N.S., Freed E.O., van Engelenburg S.B. Genomic tagging of endogenous human ESCRT-I complex preserves ESCRT-mediated membrane-remodeling functions. *J. Biol. Chem*. 2019; 294(44): 16266–81. https://doi.org/10.1074/jbc.RA119.009372
29. Meusser B., Purfuerst B., Luft F.C. HIV-1 Gag release from yeast reveals ESCRT interaction with the Gag N-terminal protein region. *J. Biol. Chem*. 2020; 295(52): 17950–72. https://doi.org/10.1074/jbc.RA120.014710
30. Hadpech S., Moonmuang S., Chupradit K., Yasamut U., Tayapiwatana C. Updating on roles of HIV intrinsic factors: a review of their antiviral mechanisms and emerging functions. *Intervirology*. 2022; 65(2): 67–79. https://doi.org/10.1159/000519241
31. Gerber P.P., Cabrini M., Jancic C., Paoletti L., Banchio C., von Bilderling C., et al. Rab27a controls HIV-1 assembly by regulating plasma membrane levels of phosphatidylinositol 4,5-bisphosphate. *J. Cell Biol*. 2015; 209(3): 435–52. https://doi.org/10.1083/jcb.201409082
32. Teow S.Y., Nordin A.C., Ali S.A., Khoo A.S. Exosomes in human immunodeficiency virus type I pathogenesis: threat or opportunity? *Adv. Virol*. 2016; 2016: 9852494. https://doi.org/10.1155/2016/9852494
33. Chiozzini C., Arenaccio C., Olivetta E., Anticoli S., Manfredi F., Ferrantelli F., et al. Trans-dissemination of exosomes from HIV-1-infected cells fosters both HIV-1 trans-infection in resting CD4+ T lymphocytes and reactivation of the HIV-1 reservoir. *Arch. Virol*. 2017; 162(9): 2565–77. https://doi.org/10.1007/s00705-017-3391-4
34. Hayes C.N., Zhang Y., Makokha G.N., Hasan M.Z., Omokoko M.D., Chayama K. Early events in hepatitis B virus infection: From the cell surface to the nucleus. *J. Gastroenterol. Hepatol*. 2016; 31(2): 302–9. https://doi.org/10.1111/jgh.13175
35. Lin Y., Wu C., Wang X., Kemper T., Squire A., Gunzer M., et al. Hepatitis B virus is degraded by autophagosome-lysosome fusion mediated by Rab7 and related components. *Protein Cell*. 2019; 10(1): 60–6. https://doi.org/10.1007/s13238-018-0555-2
36. Chou S.F., Tsai M.L., Huang J.Y., Chang Y.S., Shih C. The dual role of an ESCRT-0 component HGS in HBV transcription and naked capsid secretion. *PLoS Pathog*. 2015; 11(10): e1005123. https://doi.org/10.1371/journal.ppat.1005123
37. Prange R. Hepatitis B virus movement through the hepatocyte: An update. *Biol. Cell*. 2022; 114(12): 325–48. https://doi.org/10.1111/boc.202200060
38. Wu Q., Glitscher M., Tonnemacher S., Schollmeier A., Raupach J., Zahn T., et al. Presence of intact hepatitis B virions in exosomes. *Cell Mol. Gastroenterol. Hepatol*. 2023; 15(1): 237–59. https://doi.org/10.1016/j.jcmgh.2022.09.012
39. van der Ree M.H., Jansen L., Kruize Z., van Nuenen A.C., van Dort K.A., Takkenberg R.B., et al. Plasma MicroRNA levels are associated with hepatitis B e antigen status and treatment response in chronic hepatitis B patients. *J. Infect. Dis*. 2017; 215(9): 1421–9. https://doi.org/10.1093/infdis/jix140
40. Wang D., Huang T., Ren T., Liu Q., Zhou Z., Ge L., et al. Identification of blood exosomal miRNA-1246, miRNA-150-5p, miRNA-5787 and miRNA-8069 as sensitive biomarkers for hepatitis B virus infection. *Clin. Lab*. 2022; 68(2). https://doi.org/10.7754/Clin.Lab.2021.210415
41. Ninomiya M., Inoue J., Krueger E.W., Chen J., Cao H., Masamune A., et al. The exosome-associated tetraspanin CD63 contributes to the efficient assembly and infectivity of the hepatitis B virus. *Hepatol. Commun*. 2021; 5(7): 1238–51. https://doi.org/10.1002/hep4.1709
42. Kakizaki M., Yamamoto Y., Yabuta S., Kurosaki N., Kagawa T., Kotani A. The immunological function of extracellular vesicles in hepatitis B virus-infected hepatocytes. *PLoS One*. 2018; 13(12): e0205886. https://doi.org/10.1371/journal.pone.0205886
43. Wang C., Liu J., Yan Y., Tan Y. Role of exosomes in chronic liver disease development and their potential clinical applications. *J. Immunol. Res*. 2022; 2022: 1695802. https://doi.org/10.1155/2022/1695802
44. Bunz M., Ritter M., Schindler M. HCV egress – unconventional secretion of assembled viral particles. *Trends Microbiol*. 2022; 30(4): 364–78. https://doi.org/10.1016/j.tim.2021.08.005
45. Kulhanek K.R., Roose J.P., Rubio I. Regulation of the small GTPase Ras and its relevance to human disease. *Methods. Mol. Biol*. 2021; 2262: 19–43. https://doi.org/10.1007/978-1-0716-1190-6_2
46. Elgner F., Hildt E., Bender D. Relevance of Rab proteins for the life cycle of hepatitis C virus. *Front. Cell Dev. Biol*. 2018; 6: 166. https://doi.org/10.3389/fcell.2018.00166
47. Ahmed I., Akram Z., Iqbal H.M.N., Munn A.L. The regulation of Endosomal Sorting Complex Required for Transport and accessory proteins in multivesicular body sorting and enveloped viral budding – an overview. *Int. J. Biol. Macromol*. 2019; 127: 1–11. https://doi.org/10.1016/j.ijbiomac.2019.01.015
48. Corless L., Crump C.M., Griffin S.D., Harris M. Vps4 and the ESCRT-III complex are required for the release of infectious hepatitis C virus particles. *J. Gen. Virol*. 2010; 91(Pt. 2): 362–72. https://doi.org/10.1099/vir.0.017285-0
49. Li C., Gao Z., Cui Z., Liu Z., Bian Y., Sun H., et al. Deubiquitylation of Rab35 by USP32 promotes the transmission of imatinib resistance by enhancing exosome secretion in gastrointestinal stromal tumours. *Oncogene*. 2023; 42(12): 894–910. https://doi.org/10.1038/s41388-023-02600-1
50. Kumar S., Barouch-Bentov R., Xiao F., Schor S., Pu S., Biquand E., et al. MARCH8 ubiquitinates the hepatitis C virus nonstructural 2 protein and mediates viral envelopment. *Cell Rep*. 2019; 26(7): 1800–14.e5. https://doi.org/10.1016/j.celrep.2019.01.075
51. Tamai K., Shiina M., Tanaka N., Nakano T., Yamamoto A., Kondo Y., et al. Regulation of hepatitis C virus secretion by the Hrs-dependent exosomal pathway. *Virology*. 2012; 422(2): 377–85. https://doi.org/10.1016/j.virol.2011.11.009

52. Tamai K., Tanaka N., Nakano T., Kakazu E., Kondo Y., Inoue J., et al. Exosome secretion of dendritic cells is regulated by Hrs, an ESCRT-0 protein. *Biochem. Biophys. Res. Commun*. 2010; 399(3): 384–90. https://doi.org/10.1016/j.bbrc.2010.07.083
53. Barouch-Bentov R., Neveu G., Xiao F., Beer M., Bekerman E., Schor S., et al. Hepatitis C virus proteins interact with the Endosomal Sorting Complex Required for Transport (ESCRT) machinery via ubiquitination to facilitate viral envelopment. *mBio*. 2016; 7(6): e01456-16. https://doi.org/10.1128/mBio.01456-16
54. Younossi Z., Tacke F., Arrese M., Chander Sharma B., Mostafa I., Bugianesi E., et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology*. 2019; 69(6): 2672–82. https://doi.org/10.1002/hep.30251
55. Newman L.A., Muller K., Rowland A. Circulating cell-specific extracellular vesicles as biomarkers for the diagnosis and monitoring of chronic liver diseases. *Cell Mol. Life Sci*. 2022; 79(5): 232. https://doi.org/10.1007/s00018-022-04256-8
56. Li J., Liu H., Mauer A.S., Lucien F., Raiter A., Bandla H., et al. Characterization of cellular sources and circulating levels of extracellular vesicles in a dietary murine model of nonalcoholic steatohepatitis. *Hepatol. Commun*. 2019; 3(9): 1235–49. https://doi.org/10.1002/hep4.1404
57. Shah R., Patel T., Freedman J.E. Circulating extracellular vesicles in human disease. *N. Engl. J. Med*. 2018; 379(10): 958–66. https://doi.org/10.1056/NEJMra1704286
58. Ramakrishnaiah V., Thumann C., Fofana I., Habersetzer F., Pan Q., de Ruiter P.E., et al. Exosome-mediated transmission of hepatitis C virus between human hepatoma Huh7.5 cells. *Proc. Natl Acad. Sci. USA*. 2013; 110(32): 13109–13. https://doi.org/10.1073/pnas.1221899110
59. Bukong T.N., Momen-Heravi F., Kodys K., Bala S., Szabo G. Exosomes from hepatitis C infected patients transmit HCV infection and contain replication competent viral RNA in complex with Ago2-miR122-HSP90. *PLoS Pathog*. 2014; 10(10): e1004424. https://doi.org/10.1371/journal.ppat.1004424
60. Dreux M., Garaigorta U., Boyd B., Décembre E., Chung J., Whitten-Bauer C., et al. Short-range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity. *Cell Host. Microbe*. 2012; 12(4): 558–70. https://doi.org/10.1016/j.chom.2012.08.010
61. Zhang S., Kodys K., Babcock G.J., Szabo G. CD81/CD9 tetraspanins aid plasmacytoid dendritic cells in recognition of hepatitis C virus-infected cells and induction of interferon-alpha. *Hepatology*. 2013; 58(3): 940–9. https://doi.org/10.1002/hep.25827
62. Conde-Vancells J., Rodriguez-Suarez E., Embade N., Gil D., Matthiesen R., Valle M., et al. Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. *J. Proteome. Res*. 2008; 7(12): 5157–66. https://doi.org/10.1021/pr8004887
63. Properzi F., Logozzi M., Fais S. Exosomes: the future of biomarkers in medicine. *Biomark. Med*. 2013; 7(5): 769–78. https://doi.org/10.2217/bmm.13.63
64. Szabo G., Momen-Heravi F. Extracellular vesicles in liver disease and potential as biomarkers and therapeutic targets. *Nat. Rev. Gastroenterol. Hepatol*. 2017; 14(8): 455–66. https://doi.org/10.1038/nrgastro.2017.71
65. Borrelli D.A., Yankson K., Shukla N., Vilanilam G., Ticer T., Wolfram J. Extracellular vesicle therapeutics for liver disease. *J. Control. Release*. 2018; 273: 86–98. https://doi.org/10.1016/j.jconrel.2018.01.022
66. Lin D., Reddy V., Osman H., Lopez A., Koksal A.R., Rhadhi S.M., et al. Additional inhibition of Wnt/β-catenin signaling by metformin in DAA treatments as a novel therapeutic strategy for HCV-Infected patients. *Cells*. 2021; 10(4): 790. https://doi.org/10.3390/cells10040790
67. McVey M.J., Kuebler W.M. Extracellular vesicles: biomarkers and regulators of vascular function during extracorporeal circulation. *Oncotarget*. 2018; 9(98): 37229–51. https://doi.org/10.18632/oncotarget.26433
68. Fendl B., Weiss R., Eichhorn T., Linsberger I., Afonyushkin T., Puhm F., et al. Extracellular vesicles are associated with C-reactive protein in sepsis. *Sci. Rep*. 2021; 11(1): 6996. https://doi.org/10.1038/s41598-021-86489-4
69. U.S. National Library of Medicine. Available at: https://clinicaltrials.gov/
70. Feng Y., Wang A.T., Jia H.H., Zhao M., Yu H. A brief analysis of mesenchymal stem cells as biological drugs for the treatment of Acute-on-Chronic Liver Failure (ACLF): safety and potency. *Curr. Stem. Cell Res. Ther*. 2020; 15(3): 202–10. https://doi.org/10.2174/1574888X15666200101124317
71. Qian X., Xu C., Fang S., Zhao P., Wang Y., Liu H., et al. Exosomal microRNAs derived from umbilical mesenchymal stem cells inhibit hepatitis C virus infection. *Stem. Cells Transl. Med*. 2016; 5(9): 1190–203. https://doi.org/10.5966/sctm.2015-0348
72. Khatun M., Ray R.B. Mechanisms underlying hepatitis C virus-associated hepatic fibrosis. *Cells*. 2019; 8(10): 1249. https://doi.org/10.3390/cells8101249
73. Devhare P.B., Sasaki R., Shrivastava S., Di Bisceglie A.M., Ray R., Ray R.B. Exosome-mediated intercellular communication between hepatitis C virus-infected hepatocytes and hepatic stellate cells. *J. Virol*. 2017; 91(6): e02225-16. https://doi.org/10.1128/JVI.02225-16
74. Kim J.H., Lee C.H., Lee S.W. Exosomal transmission of microRNA from HCV replicating cells stimulates transdifferentiation in hepatic stellate cells. *Mol. Ther. Nucleic. Acids*. 2019; 14: 483–97. https://doi.org/10.1016/j.omtn.2019.01.006
75. Bruno S., Pasquino C., Herrera Sanchez M.B., Tapparo M., Figliolini F., Grange C., et al. HLSC-derived extracellular vesicles attenuate liver fibrosis and inflammation in a murine model of non-alcoholic steatohepatitis. *Mol. Ther*. 2020; 28(2): 479–89. https://doi.org/10.1016/j.ymthe.2019.10.016
76. Chiabotto G., Ceccotti E., Tapparo M., Camussi G., Bruno S. Human liver stem cell-derived extracellular vesicles target hepatic stellate cells and attenuate their pro-fibrotic phenotype. *Front. Cell Dev. Biol*. 2021; 9: 777462. https://doi.org/10.3389/fcell.2021.777462
77. Rong X., Liu J., Yao X., Jiang T., Wang Y., Xie F. Human bone marrow mesenchymal stem cells-derived exosomes alleviate liver fibrosis through the Wnt/β-catenin pathway. *Stem. Cell Res. Ther*. 2019; 10(1): 98. https://doi.org/10.1186/s13287-019-1204-2
78. Kim J., Lee C., Shin Y., Wang S., Han J., Kim M., et al. sEVs from tonsil-derived mesenchymal stromal cells alleviate activation of hepatic stellate cells and liver fibrosis through miR-486-5p. *Mol. Ther*. 2021; 29(4): 1471–86. https://doi.org/10.1016/j.ymthe.2020.12.025
79. Du Z., Wu T., Liu L., Luo B., Wei C. Extracellular vesicles-derived miR-150-5p secreted by adipose-derived mesenchymal stem cells inhibits CXCL1 expression to attenuate hepatic fibrosis. *J. Cell Mol. Med*. 2021; 25(2): 701–15. https://doi.org/10.1111/jcmm.16119
80. Chen L., Chen R., Kemper S., Cong M., You H., Brigstock D.R. Therapeutic effects of serum extracellular vesicles in liver fibrosis. *J. Extracell. Vesicles*. 2018; 7(1): 1461505. https://doi.org/10.1080/20013078.2018.1461505
81. Bruno S., Chiabotto G., Camussi G. Extracellular vesicles: a therapeutic option for liver fibrosis. *Int. J. Mol. Sci*. 2020; 21(12): 4255. https://doi.org/10.3390/ijms21124255
82. Hwang S., Yang Y.M. Exosomal microRNAs as diagnostic and therapeutic biomarkers in non-malignant liver diseases. *Arch. Pharm. Res*. 2021; 44(6): 574–87. https://doi.org/10.1007/s12272-021-01338-2
83. Zhou Y., Wang X., Sun L., Zhou L., Ma T.C., Song L., et al. Toll-like receptor 3-activated macrophages confer anti-HCV activity to hepatocytes through exosomes. *FASEB J*. 2016; 30(12): 4132–40. https://doi.org/10.1096/fj.201600696R
84. Fasbender F., Widera A., Hengstler J.G., Watzl C. Natural killer cells and liver fibrosis. *Front. Immunol*. 2016; 7: 19. https://doi.org/10.3389/fimmu.2016.00019
85. Neviani P., Wise P.M., Murtadha M., Liu C.W., Wu C.H., Jong A.Y., et al. Natural killer-derived exosomal miR-186 inhibits neuroblastoma growth and immune escape mechanisms. *Cancer Res*. 2019; 79(6): 1151–64. https://doi.org/10.1158/0008-5472.CAN-18-0779
86. Wang L., Wang Y., Quan J. Exosomes derived from natural killer cells inhibit hepatic stellate cell activation and liver fibrosis. *Hum. Cell*. 2020; 33(3): 582–9. https://doi.org/10.1007/s13577-020-00371-5
87. Target Scan Human. Whitehead Institute for Biomedical Research. Available at: http://www.targetscan.org
88. Wang L., Wang Y., Quan J. Exosomal miR-223 derived from natural killer cells inhibits hepatic stellate cell activation by suppressing autophagy. *Mol. Med*. 2020; 26(1): 81. https://doi.org/10.1186/s10020-020-00207-w
89. Ye H.L., Zhang J.W., Chen X.Z., Wu P.B., Chen L., Zhang G. Ursodeoxycholic acid alleviates experimental liver fibrosis involving inhibition of autophagy. *Life Sci*. 2020; 242: 117175. https://doi.org/10.1016/j.lfs.2019.117175
90. Zhang Y., Hua L., Lin C., Yuan M., Xu W., Raj D.A., et al. Pi-en-Tze-Huang alleviates CCl4-induced liver fibrosis through the inhibition of HSC autophagy and the TGF-β1/Smad2 pathway.

Front. Pharmacol. 2022; 13: 937484. https://doi.org/10.3389/fphar.2022.937484
91. Avalos P.N., Forsthoefel D.J. An emerging frontier in intercellular communication: extracellular vesicles in regeneration. *Front. Cell Dev. Biol*. 2022; 10: 849905. https://doi.org/10.3389/fcell.2022.849905
92. Verweij F.J., Revenu C., Arras G., Dingli F., et al. Live tracking of inter-organ communication by endogenous exosomes in vivo. *Dev. Cell*. 2019; 48(4): 573–89.e4. https://doi.org/10.1016/j.devcel.2019.01.004
93. Matsumoto A., Takahashi Y., Nishikawa M., Sano K., Morishita M., Charoenviriyakul C., et al. Role of phosphatidylserine-derived negative surface charges in the recognition and uptake of intravenously injected B16BL6-derived exosomes by macrophages. *J. Pharm. Sci*. 2017; 106(1): 168–75. https://doi.org/10.1016/j.xphs.2016.07.022
94. Wu R., Fan X., Wang Y., Shen M., Zheng Y., Zhao S., et al. Mesenchymal stem cell-derived extracellular vesicles in liver immunity and therapy. *Front. Immunol*. 2022; 13: 833878. https://doi.org/10.3389/fimmu.2022.833878
95. Schorey J.S., Harding C.V. Extracellular vesicles and infectious diseases: new complexity to an old story. *J. Clin. Invest*. 2016; 126(4): 1181–9. https://doi.org/10.1172/JCI81132
96. Yao Z., Qiao Y., Li X., Chen J., Ding J., Bai L., et al. Exosomes exploit the virus entry machinery and pathway to transmit alpha interferon-induced antiviral activity. *J. Virol*. 2018; 92(24): e01578-18. https://doi.org/10.1128/JVI.01578-18
97. Hassanpour M., Rezaie J., Nouri M., Panahi Y. The role of extracellular vesicles in COVID-19 virus infection. *Infect. Genet. Evol*. 2020; 85: 104422. https://doi.org/10.1016/j.meegid.2020.104422
98. Earnest J.T., Hantak M.P., Li K., McCray P.B. Jr., Perlman S., Gallagher T. The tetraspanin CD9 facilitates MERS-coronavirus entry by scaffolding host cell receptors and proteases. *PLoS Pathog*. 2017; 13(7): e1006546. https://doi.org/10.1371/journal.ppat.1006546
99. Barberis E., Vanella V.V., Falasca M., Caneapero V., Cappellano G., Raineri D., et al. Circulating exosomes are strongly involved in SARS-CoV-2 infection. *Front. Mol. Biosci*. 2021; 8: 632290. https://doi.org/10.3389/fmolb.2021.632290
100. Bansal S., Perincheri S., Fleming T., Poulson C., Tiffany B., Bremner R.M., et al. Cutting edge: circulating exosomes with COVID spike protein are induced by BNT162b2 (Pfizer-BioNTech) vaccination prior to development of antibodies: a novel mechanism for immune activation by mRNA vaccines. *J. Immunol*. 2021; 207(10): 2405–10. https://doi.org/10.4049/jimmunol.2100637
101. Sur S., Khatun M., Steele R., Isbell T.S., Ray R., Ray R.B. Exosomes from COVID-19 patients carry tenascin-C and fibrinogen-β in triggering inflammatory signals in cells of distant organ. *Int. J. Mol. Sci*. 2021; 22(6): 3184. https://doi.org/10.3390/ijms22063184
102. Mills J.T., Schwenzer A., Marsh E.K., Edwards M.R., Sabroe I., Midwood K.S., et al. Airway epithelial cells generate pro-inflammatory tenascin-C and small extracellular vesicles in response to TLR3 stimuli and rhinovirus infection. *Front. Immunol*. 2019; 10: 1987. https://doi.org/10.3389/fimmu.2019.01987
103. Gupta A., Madhavan M.V., Sehgal K., Nair N., Mahajan S., Sehrawat T.S., et al. Extrapulmonary manifestations of COVID-19. *Nat. Med*. 2020; 26(7): 1017–32. https://doi.org/10.1038/s41591-020-0968-3
104. Kwon Y., Nukala S.B., Srivastava S., Miyamoto H., Ismail N.I., Jousma J., et al. Detection of viral RNA fragments in human iPSC cardiomyocytes following treatment with extracellular vesicles from SARS-CoV-2 coding sequence overexpressing lung epithelial cells. *Stem. Cell Res. Ther*. 2020; 11(1): 514. https://doi.org/10.1186/s13287-020-02033-7
105. Wang J., Chen S., Bihl J. Exosome-mediated transfer of ACE2 (Angiotensin-Converting Enzyme 2) from endothelial progenitor cells promotes survival and function of endothelial cell. *Oxid. Med. Cell Longev*. 2020; 2020: 4213541. https://doi.org/10.1155/2020/4213541
106. Cocozza F., Névo N., Piovesana E., Lahaye X., Buchrieser J., Schwartz O., et al. Extracellular vesicles containing ACE2 efficiently prevent infection by SARS-CoV-2 Spike protein-containing virus. *J. Extracell. Vesicles*. 2020; 10(2): e12050. https://doi.org/10.1002/jev2.12050
107. El-Shennawy L., Hoffmann A.D., Dashzeveg N.K., McAndrews K.M., Mehl P.J., Cornish D., et al. Circulating ACE2-expressing extracellular vesicles block broad strains of SARS-CoV-2. *Nat. Commun*. 2022; 13(1): 405. https://doi.org/10.1038/s41467-021-27893-2
108. Ching K.L., de Vries M., Gago J., Dancel-Manning K., Sall J., Rice W.J., et al. ACE2-containing defensosomes serve as decoys to inhibit SARS-CoV-2 infection. *PLoS Biol*. 2022; 20(9): e3001754. https://doi.org/10.1371/journal.pbio.3001754
109. Song J.W., Lam S.M., Fan X., Cao W.J., Wang S.Y., Tian H., et al. Omics-driven systems interrogation of metabolic dysregulation in COVID-19 pathogenesis. *Cell Metab*. 2020; 32(2): 188–202.e5. https://doi.org/10.1016/j.cmet.2020.06.016
110. Shenoy G.N., Loyall J., Berenson C.S., Kelleher R.J. Jr., Iyer V., Balu-Iyer S.V., et al. Sialic acid-dependent inhibition of T cells by exosomal ganglioside GD3 in ovarian tumor microenvironments. *J. Immunol*. 2018; 201(12): 3750–8. https://doi.org/10.4049/jimmunol.1801041
111. Schnaar R.L. The biology of gangliosides. *Adv. Carbohydr. Chem. Biochem*. 2019; 76: 113–48. https://doi.org/10.1016/bs.accb.2018.09.002
112. Fantini J., Chahinian H., Yahi N. Leveraging coronavirus binding to gangliosides for innovative vaccine and therapeutic strategies against COVID-19. *Biochem. Biophys. Res. Commun*. 2021; 538: 132–6. https://doi.org/10.1016/j.bbrc.2020.10.015
113. Hall M.W., Joshi I., Leal L., Ooi E.E. Immune immunomodulation in coronavirus disease 2019 (COVID-19): strategic considerations for personalized therapeutic intervention. *Clin. Infect. Dis*. 2022; 74(1): 144–8. https://doi.org/10.1093/cid/ciaa904
114. Holnthoner W., Bonstingl C., Hromada C., Muehleder S., Zipperle J., Stojkovic S., et al. Endothelial cell-derived extracellular vesicles size-dependently exert procoagulant activity detected by thromboelastometry. *Sci. Rep*. 2017; 7(1): 3707. https://doi.org/10.1038/s41598-017-03159-0
115. Balbi C., Burrello J., Bolis S., Lazzarini E., Biemmi V., Pianezzi E., et al. Circulating extracellular vesicles are endowed with enhanced procoagulant activity in SARS-CoV-2 infection. *EBioMedicine*. 2021; 67: 103369. https://doi.org/10.1016/j.ebiom.2021.103369
116. Cappellano G., Raineri D., Rolla R., Giordano M., Puricelli C., Vilardo B., et al. Circulating platelet-derived extracellular vesicles are a hallmark of SARS-Cov-2 infection. *Cells*. 2021; 10(1): 85. https://doi.org/10.3390/cells10010085
117. Tahyra A.S.C., Calado R.T., Almeida F. The role of extracellular vesicles in COVID-19 pathology. *Cells*. 2022; 11(16): 2496. https://doi.org/10.3390/cells11162496
118. Yang C.W., Chen R.D., Zhu Q.R., Han S.J., Kuang M.J. Efficacy of umbilical cord mesenchymal stromal cells for COVID-19: A systematic review and meta-analysis. *Front. Immunol*. 2022; 13: 923286. https://doi.org/10.3389/fimmu.2022.923286
119. Tan M.I., Alfarafisa N.M., Septiani P., Barlian A., Firmansyah M., Faizal A., et al. Potential cell-based and cell-free therapy for patients with COVID-19. *Cells*. 2022; 11(15): 2319. https://doi.org/10.3390/cells11152319
120. Bari E., Ferrarotti I., Saracino L., Perteghella S., Torre M.L., Richeldi L., et al. Mesenchymal stromal cell secretome for post-COVID-19 pulmonary fibrosis: a new therapy to treat the long-term lung sequelae? *Cells*. 2021; 10(5): 1203. https://doi.org/10.3390/cells10051203
121. Gardin C., Ferroni L., Chachques J.C., Zavan B. Could mesenchymal stem cell-derived exosomes be a therapeutic option for critically ill COVID-19 patients? *J. Clin. Med*. 2020; 9(9): 2762. https://doi.org/10.3390/jcm9092762
122. Perets N., Hertz S., London M., Offen D. Intranasal administration of exosomes derived from mesenchymal stem cells ameliorates autistic-like behaviors of BTBR mice. *Mol. Autism*. 2018; 9: 57. https://doi.org/10.1186/s13229-018-0240-6
123. Elahi F.M., Farwell D.G., Nolta J.A., Anderson J.D. Preclinical translation of exosomes derived from mesenchymal stem/stromal cells. *Stem. Cells*. 2020; 38(1): 15–21. https://doi.org/10.1002/stem.3061
124. Allan D., Tieu A., Lalu M., Burger D. Mesenchymal stromal cell-derived extracellular vesicles for regenerative therapy and immune modulation: Progress and challenges toward clinical application. *Stem. Cells Transl. Med*. 2020; 9(1): 39–46. https://doi.org/10.1002/sctm.19-0114
125. Dinh P.C., Paudel D., Brochu H., Popowski K.D., Gracieux M.C., Cores J., et al. Inhalation of lung spheroid cell secretome and exosomes promotes lung repair in pulmonary fibrosis. *Nat. Commun*. 2020; 11(1): 1064. https://doi.org/10.1038/s41467-020-14344-7

126. Cocozza F., Névo N., Piovesana E., Lahaye X., Buchrieser J., Schwartz O., et al. Extracellular vesicles containing ACE2 efficiently prevent infection by SARS-CoV-2 Spike protein-containing virus. *J. Extracell. Vesicles*. 2020; 10(2): e12050. https://doi.org/10.1002/jev2.12050
127. Inal J.M. Decoy ACE2-expressing extracellular vesicles that competitively bind SARS-CoV-2 as a possible COVID-19 therapy. *Clin. Sci. (Lond)*. 2020; 134(12): 1301–4. https://doi.org/10.1042/CS20200623
128. Askenase P.W. COVID-19 therapy with mesenchymal stromal cells (MSC) and convalescent plasma must consider exosome involvement: Do the exosomes in convalescent plasma antagonize the weak immune antibodies? *J. Extracell. Vesicles*. 2020; 10(1): e12004. https://doi.org/10.1002/jev2.12004
129. Rezabakhsh A., Mahdipour M., Nourazarian A., Habibollahi P., Sokullu E., Avci Ç.B., et al. Application of exosomes for the alleviation of COVID-19-related pathologies. *Cell Biochem. Funct*. 2022; 40(5): 430–8. https://doi.org/10.1002/cbf.3720
130. Sengupta V., Sengupta S., Lazo A., Woods P., Nolan A., Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. *Stem. Cells Dev*. 2020; 29(12): 747–54. https://doi.org/10.1089/scd.2020.0080
131. Kuate S., Cinatl J., Doerr H.W., Uberla K. Exosomal vaccines containing the S protein of the SARS coronavirus induce high levels of neutralizing antibodies. *Virology*. 2007; 362(1): 26–37. https://doi.org/10.1016/j.virol.2006.12.011
132. Sharma K., Koirala A., Nicolopoulos K., Chiu C., Wood N., Britton P.N. Vaccines for COVID-19: Where do we stand in 2021? *Paediatr. Respir. Rev*. 2021; 39: 22–31. https://doi.org/10.1016/j.prrv.2021.07.001
133. Wang Z., Popowski K.D., Zhu D., de Juan Abad B.L., Wang X., Liu M., et al. Exosomes decorated with a recombinant SARS-CoV-2 receptor-binding domain as an inhalable COVID-19 vaccine. *Nat. Biomed. Eng*. 2022; 6(7): 791–805. https://doi.org/10.1038/s41551-022-00902-5
134. Altan-Bonnet N. Extracellular vesicles are the Trojan horses of viral infection. *Curr. Opin. Microbiol*. 2016; 32: 77–81. https://doi.org/10.1016/j.mib.2016.05.004
135. Badierah R.A., Uversky V.N., Redwan E.M. Dancing with Trojan horses: an interplay between the extracellular vesicles and viruses. *J. Biomol. Struct. Dyn*. 2021; 39(8): 3034–60. https://doi.org/10.1080/07391102.2020.1756409
136. Sun L., Wang X., Zhou Y., Zhou R.H., Ho W.Z., Li J.L. Exosomes contribute to the transmission of anti-HIV activity from TLR3-activated brain microvascular endothelial cells to macrophages. *Antiviral. Res*. 2016; 134: 167–71. https://doi.org/10.1016/j.antiviral.2016.07.013
137. Welch J.L., Kaddour H., Schlievert P.M., Stapleton J.T., Okeoma C.M. Semen exosomes promote transcriptional silencing of HIV-1 by disrupting NF-κB/Sp1/Tat circuitry. *J. Virol*. 2018; 92(21): e00731-18. https://doi.org/10.1128/JVI.00731-18
138. Chen J., Li C., Li R., Chen H., Chen D., Li W. Exosomes in HIV infection. *Curr. Opin. HIV AIDS*. 2021; 16(5): 262–70. https://doi.org/10.1097/COH.0000000000000694
139. Abraham A., Krasnodembskaya A. Mesenchymal stem cell-derived extracellular vesicles for the treatment of acute respiratory distress syndrome. *Stem. Cells. Transl. Med*. 2020; 9(1): 28–38. https://doi.org/10.1002/sctm.19-0205
140. Popowski K.D., Dinh P.C., George A., Lutz H., Cheng K. Exosome therapeutics for COVID-19 and respiratory viruses. *View (Beijing)*. 2021; 2(3): 20200186. https://doi.org/10.1002/VIW.20200186
141. Rangel-Ramírez V.V., González-Sánchez H.M., Lucio-García C. Exosomes: from biology to immunotherapy in infectious diseases. *Infect. Dis. (Lond)*. 2023; 55(2): 79–107. https://doi.org/10.1080/23744235.2022.2149852