Current Applications and Challenges of Exosome in COVID-19: A Review

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Abstract. The exosome is a nano-extracellular vesicle secreted by the cell, encasing proteins, nucleic acids, and lipids, among others. Its specific structural composition gives it a role in the medical field. It may act as biomarkers, vaccines and drug carriers. Since the outbreak of COVID-19, the rate of infection has increased dramatically worldwide, as it targets ACE2 in a wide range of human cells, posing a huge threat of loss to human and global health as well as the economy. Exosomes are highly investigated as a highly promising candidate when exploring prevention and treatment against COVID-19. Many experiments have been performed to demonstrate the potential of exosomes in a sufficiently well-documented manner. In this review, we summarize the potential of exosomes in the diagnosis, prevention and treatment of COVID-19, and analyze the problems, with the aim of providing valuable directions for further research on exosomes in the future.

Keywords: COVID-19, Exosomes, Diagnosis, Prevention, Treatment.

1. Introduction

Exosomes are membrane-encapsulated nano-extracellular vesicles secreted by the cell exocytosis, which usually contain biomolecules such as proteins, nucleic acids, and lipids [1, 2]. Almost all cells secrete exosomes in both physiological and pathological conditions and act as communication mediators capable of transmitting cellular cargoes such as metabolites, functional proteins, and nucleic acids to recipient cells and functioning in intercellular communication [3]. Thus, exosomes usually have a non-negligible role in the physiology and pathology of organisms [4]. Often, exosomes secreted by different cells contain proteins, lipids, and RNA specific to the cell of origin. The detection of cargo contents can provide research directions for disease detection and treatment. Researchers have identified these roles and opened up their application in the field of medical and pharmacological research [5]. Being a cell-derived nanovesicle, its specific immunogenicity and molecular transfer function make exosomes promising in tumor immunotherapy [6]. Exosomes have also been shown to be active in neural communication, reproductive development, homeostasis and maturation, and cell proliferation [4]. The clinical applications of exosomes can be mainly applied and used as biomarkers, drug delivery vehicles, cell-free therapeutic agents, and tumor vaccines [7].

Coronavirus disease 2019 (COVID-19) is caused by SARS-Cov-2 infection and affects primarily the respiratory system but also other organs. It spreads globally at an alarming rate after its initial detection and has a high pathogenicity and lethality rate [8-10]. Globally, COVID-19 has taken a great toll on human safety and health, daily life, and the global public health economy [9]. The disease is caused by SARS-Cov-2, a membrane-enveloped positive-stranded single-stranded RNA virus, which is a type of coronavirus. The SARS-Cov-2 consists of four structural proteins, spike (S) envelope (E), membrane (M), and nucleocapsid (N) proteins, of which the spike protein plays a major role in infection. The spike protein consists of two subunits S1 and S2, S1 is responsible for binding to the receptor of the host cell and S2 is responsible for enabling the binding of the cell membrane to the viral membrane. SARS-Cov-2 can infect a wide range of cells, especially humans, the main receptor mediating the infection is ACE2 and this receptor is expressed in multiple locations in the body [10]. The specific infection process is internalized into the cell by the cytosolic enzyme furin catalyzing the cleavage of the virus at the S1/S1 site on the stinger protein and the TMPRSS2 initiation allowing it to bind to ACE2 of the host cell. After infection, the virus usually regulates various inflammatory factors, resulting in an immune response in the host and, depending on the

severity of the disease, a decrease in NK, lymphatic T cells, etc., causing severe damage to all body functions of the host [10, 11].

Due to the tremendous impact of COVID-19 on humans, many scientists are involved in entering the research of COVID-19 control. For now, the most widely used and effective COVID-19 tests worldwide are immunoenzymatic assay, rapid antigen and antibody assays and RT-PCR-based molecular detection techniques [12]. According to the WHO report, as of May 6, 2022, there are more than three hundred COVID-19 vaccines in the preclinical or clinical development stage, followed by ten as well as approved by WHO to be used worldwide [13]. Liposomes can be used as a delivery system to bind to mRNA as an injectable COVID-19 vaccine, which protects mRNA from degradation by enzymes in the blood circulation. Currently, liposome-based mRNA COVID-19 vaccines have been developed and used in millions of people [14]. Adenovirus-based vaccines are used more widely in developing countries, while mRNA vaccines are used in developed countries. Vaccination rates show global inequalities. Improvements are needed in vaccine stability, neutralizing antibody titers, and duration of protection. Current vaccines have safety concerns and are less effective against new strains like Omicron [13, 15]. Currently, there is evidence that only inhaled interferons, small molecules and monoclonal antibodies can be used as effective treatments in the early stages of neointimal infections. However, these drugs are expensive and in limited supply. The availability of these drugs is limited. In addition, no effective therapeutic agents have been diagnosed for COVID-19 except for three drugs, raltegravir, camostat mesylate and favipiravir, of which raltegravir is also an obstacle to its widespread use worldwide because of its high cost [11, 16].

As mentioned above, COVID-19 currently still faces many challenges in terms of vaccine safety, efficacy, and durability. Exosomes, with their low immunogenicity, high biocompatibility, and the ability to be modified and engineered for targeting and specificity [2, 17], have the potential to be used as effective alternatives as stable drug carriers or modified as vaccines or even multivalent vaccines against viral invasion and mutation. At the same time, exosomes are produced by almost all cells in the host to process cell communication, including infected cells [17], so the detection of exosomes can be used as a reinvention of the diagnostic approach, and inhibition of the production of infectious exosomes may be effective for prevention. In addition, exosomes themselves have regulatory capabilities and may be produced in large quantities, and are worthy of discussion as drugs to treat and address production volume and cost issues.

In this review, we will discuss the specific potential of exosomes in the prevention and treatment of COVID-19, while citing the current challenges in the field to provide a reference direction for further research.

2. The potential function of exosomes in the diagnosis and prevention of COVID-19

2.1. Diagnosis

Since exosomes can be secreted by virtually any cell, epithelial cells infected with SARS-Cov-2 are no exception. Exosomes secreted by this epithelial cell itself have a special proteome, including fibronectin, fibrinogen, P amyloid, and complement C1r subset. These proteins can be used as specific biomarkers in the diagnosis of covid-19 and disease tracking and judgment of patients [18, 19]. Platelet-derived extracellular vesicles (PLT-EVs) have been reported to be detected in the platelet-free plasma of patients infected with SARA-Cov-2 and, depending on the severity of COVID-19 disease, the number of PLT-EVs was significantly greater in patients than in healthy individuals [20]. COVID-19 infection induces platelets to produce platelet-derived extracellular vesicles, increasing their number, and this correlates with the poor prognosis of the infected patient [21]. Therefore, exosomes can be used not only as biomarkers to accurately detect the disease and diagnose infection, but also as a possible diagnostic and predicted tool for poor prognosis by detecting the abundance of exosomes in the blood [20]. However, the exosome only provides a new diagnostic tool, and further experimental studies will be valuable in the future in order to effectively assess.

2.2. Prevention

It has been found that SARS-Cov-2 causes infection by binding the receptor binding domain (RBD) of the S1 subunit of its S protein to angiotensin-transferase 2 (ACE2) in airway epithelial cells. It was also found that exosomes secreted by lung spheroid cells (LSC) could effectively promote the repair of damaged lungs in a mouse model. Based on these findings, RBD and LSC-exo were combined into an inhaled vaccine. Usually, people use mRNA vaccines by intramuscular injection, but studies have found that RBD-exo inhalable vaccines can induce the production of neutralizing antibodies against SARS-Cov-2, while antigen-specific secretory IgA and t cells can be induced to produce a response within the mucosal system, thus preventing virus uptake by the lung epithelium. In detail, in the hamster model, two doses of the vaccine successfully attenuated severe pneumonia while reducing the inflammatory infiltrates induced by the live virus vaccine. These results indicate that the exosome vaccine has the potential to be an effective preventive measure. Compared to vaccines that use liposomes as a carrier, LSCs-exo can act as a more targeted delivery vehicle than liposomes because their surface proteins and receptors possess the membrane characteristics of the airway epithelium, allowing them to be widely distributed and present in the lung for a long time, and to play a role in enhancing the internalization of APCs in the lung [22].

Meanwhile, in experiments in mice, transnasal administration of extracellular vesicles EVs-ACE2 encapsulated with engineered ACE2 was able to block the entry of S pseudovirus into the mucosal epithelium and could be used as an effective prophylactic means to cut off the infection pathway at the initial stage of exposure [23]. The engineered exosomes can carry the ACE2 receptor on the surface as a competitive inhibitor to bind to SARS-CoV-2 and label it, which is then recognized and cleaned by immune cells and difficult to form membrane fusion with host cells, thereby reducing the binding of the virus to host cells. This is the utilization of exosomes as nano-decoy, which can effectively reduce the spread of the virus during early viral infection [24, 25].

Currently, the COVID-19 vaccines that have been widely used are mainly based on viral vectors, especially adenovirus. However, the disadvantage of using viral vectors is that the host immune expression against covid-19 may be limited due to the inherent immunity of the viral vectors, which may lead to reduced vaccine efficacy and inability to produce long-term immunity. There is a risk that vaccines will no longer be effective when the virus mutates to produce new strains. It is known that SARS-CoV-2 has four key structural proteins, among which the S protein is the most variable, and the other three proteins are more stable and conserved. In order to avoid immune escape, the engineered exosomes were designed to use S, M, E and N as target antigens, after which NAb and T cell responses could be strongly induced. Therefore, such exosome vaccines could allow the host to acquire long-term immunity and be more adapted to immunity against variants without the effects of vaccine-induced virulence reversal and the existing immunity of the vector [26].

3. The potential of exosome in the treatment of covid-19

COVID-19 infection usually causes a cytokine storm, which is the main cause of acute respiratory distress syndrome and multi-organ failure in patients. Exosomes have been shown to have immunomodulatory roles in organisms, and it has been shown that exosomes derived from mesenchymal stem cells treated in a medium that induces exosome release can be identified and isolated and administered intravenously. These exosomes were found to suppress cytokine storm by inhibiting NK cells, CD4+, and CD8+ T cells. Moreover, the differentiation of CD4+ and CD8+ T cells can be suppressed by TFGβ that is released by exos, thereby inhibiting inflammation [27].

Exosomes also stimulate cell regeneration and functional recovery under many pathologic conditions. In particular, MSC-exo can inhibit cell fibrosis by preventing fibroblast differentiation into myofibroblasts [18]. In COVID-19 patients, many patients have symptoms of multi-organ damage, which is due to the high expression of the ACE2 receptors in multiple organs in the human body [28]. A number of studies and data have shown that MSCs from different sources have certain repair and regeneration effects for different organs and different degrees of injury [27, 28]. Sepsis is

a symptom of the high pathogenicity of SARS-CoV-2. Some studies have successfully improved the survival rate of septic mice by injecting exosomes. LPS-stimulated tumor cells can secret exosomes protecting host from sepsis [27, 29]. Therefore, This would also be a potential therapeutic approach for exosomes against COVID-19 sepsis in humans.

In addition, exosomes are often used as carriers for drug delivery due to their unique low antigenicity and high biocompatibility [26]. In the treatment of covid-19, antiviral drugs can be engineered to be encapsulated in exosomes, and due to the different sources of exosomes themselves, they can be delivered to the infected area to produce effects, effectively reducing off-target effects. Combined with the innate anti-inflammatory effect of exosomes, these engineered exosomes may be able to target SARS-Cov-19 infected cells to effectively attenuate viral replication and inhibit a series of inflammation caused by cytokine storms. Thus, it can reduce the immune response and reduce the damage to the tissues infected by the novel coronavirus [11, 27].

Moreover, viral infections may enter the pathway of exosome secretion, which means that the virus can spread to other healthy cells through exosomes secreted by the infected cells for infection. In this case, the design of an exosome bioinhibitor may be effective in preventing the spread of the virus through the exosome form, thus reducing the degree of infection [17].

4. Challenges of Exosomes-based drug development in COVID-19

At present, the application of exosomes still has a very bright prospect, but there are still several key problems to be solved in the current research. MSC-exos is considered by many people as an ideal drug for the treatment of COVID-19, which has been confirmed by many experiments and animal models. However, most of the subjects studied are in vitro models and animal models, and relevant clinical trials are still lacking at present, so the actual specificity, proficiency and safety of the experiments in the clinical setting are not yet available [17, 27]. Therefore, more experiments are needed to study and prove the clinical application of exosomes for COVID-19 [27]. More specifically, if exosomes are used as novel coronavirus drugs in humans, the corresponding treatment methods, the most appropriate dose, the correct route of administration, the condition of metabolism, and the culture system are all critical, and there is no mature set of standards at present [24, 27]. For exosome inhibitors, the targeting design of inhibitors is still imperfect, and, likely, that exosome inhibitors will unforgettably inhibit exosome biosynthesis to normal cells [17].

Due to the lack of specific mechanisms for the relationship between exosome characteristics and function, and the lack of scalable solutions for separating particular exosomes from other large entities, the contemporary purification technique and isolation method are not mature, and significant obstacles remain for the efficient and reliable isolation of large amounts of pure and specific exosomes [4, 24]. It is possible that the Morphology and integrity of exo may change, and the extract may become contaminated resulting in low purity [24]. This largely restricts the artificial production of exosomes and prevents the isolation of large amounts of high purity and specific exosomes, making it difficult to mass-produce drug candidates for design as well as potentially expensive, without actually alleviating the current global pain point of drug use for COVID-19. Additionally, if allogeneic exo is used for systemic administration, it is likely to be cleared by splenic and hepatic macrophages, thereby preventing the corresponding exosome vector from reaching these target sites [27].

5. Conclusion

In conclusion, exosomes still have a relatively large scope for development in COVID-19 prevention and treatment based on their specific biological properties. Exosomes have the potential to be effective as drug carriers, vaccines, therapeutic agents, or regulated objects to help alleviate the current problems in the prevention and treatment of COVID-19. Further clinical trials and studies will be conducted to support the effectiveness, high sensitivity, and safety of exosomes in the

diagnosis, prevention, and treatment of COVID-19 disease. At the same time facing the current problems of exosome development, after all, exosomes as the future of medical research is a very promising research direction, scientists will also take further steps to overcome these issues.

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