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Exploring the utility of extracellular vesicles in ameliorating viral infection-associated inflammation, cytokine storm and tissue damage

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ABSTRACT

Extracellular vesicles (EVs) have emerged as potential mediators of intercellular communication. EVs are nano-sized, lipid membrane–bound vesicles that contains biological information in the form of proteins, metabolites and/or nucleic acids. EVs are key regulators of tissue repair mechanisms, such as in the context of lung injuries. Recent studies suggest that EVs have the ability to repair COVID19-associated acute lung damage. EVs hold great promise for therapeutic treatments, particularly in treating a potentially fatal autoimmune response and attenuate inflammation. They are known to boost lung immunity and are involved in the pathogenesis of various lung diseases, including viral infection. EV-based immunization technology has been proven to elicit robust immune responses in many models of infectious disease, including COVID-19. The field of EV research has tremendous potential in advancing our understanding about viral infection pathogenesis, and can be translated into anti-viral therapeutic strategies.

Introduction

Extracellular vesicles (EVs) are nano-sized, phospholipid membrane–enclosed structures that can carry biological cargoes such as proteins, metabolites and nucleic acids [1]. EV cargoes may be of considerable importance for COVID-19 patients with acute lung injury caused by the SARS-CoV-2 virus [2,3]. EV-based therapies hold great potential for COVID-19–related lung injuries as they attenuate inflammation, boost immunity, and enhance tissue regeneration [3,4]. EVs share multiple similarities with viruses, and recent findings suggest that viruses use EVs for cellular communication and pathogenesis [4]. Structurally, EVs are similar to viruses [5]. They have affinity to incorporate virus-fragments, glycan components, proteins, and chunks of viral RNA [5]. EVs, depending on the proteins and genetic content they contain, play a significant role in promoting and suppressing viral infection [6,7]. Both EVs and viruses have the capacity to physically fuse with target cells by membrane endocytosis and incorporate nucleic acids such as RNA into host cells [7]. In addition, EVs and viruses can illicit immune reactions and/or attenuate inflammation responses in the host [7,8]. Lastly, EVs are surrounded by lipid membranes similar to enveloped viruses [8]. Because of the shared similarities between EV and decoding the mechanisms underlying EV–virus interactions could be translated into antiviral drug design and even vaccine development [8–10]. Recent studies have shown that EV cargo can be transferred to target cells and thus the vesicles themselves can be utilized for viral drug/vaccine delivery [10].

Extracellular vesicles and COVID19-associated cytokine storm

Mesenchymal stroma cells and their derived EVs secrete many types of cytokines and chemokines that closely interact with immune cells, including *T* cells, *B* cells, and macrophages, making EVs excellent candidates for an immunomodulation action [11,12]. Furthermore, EVs facilitate tissue regeneration by secreting interleukins and/or by releasing and attracting different growth factors such as TGF, FGF, and VEGF [12–14]. EVs may even neutralize COVID19-associated cytokine storm [14]. Another study has shown that EVs are being used for pneumonia-targeted delivery, which can improve therapeutic efficacy while reducing side effects [14]. Such technology can be useful for the diagnosis of inflammatory disease and for drug delivery, vaccine development, especially for COVID-19.

Abbreviations: EVs, extracellular vesicles; VEGF, vascular endothelial growth factor; ACE2, angiotensin-converting enzyme; TGFβ, transforming growth factor beta; BBB, blood-brain barrier.

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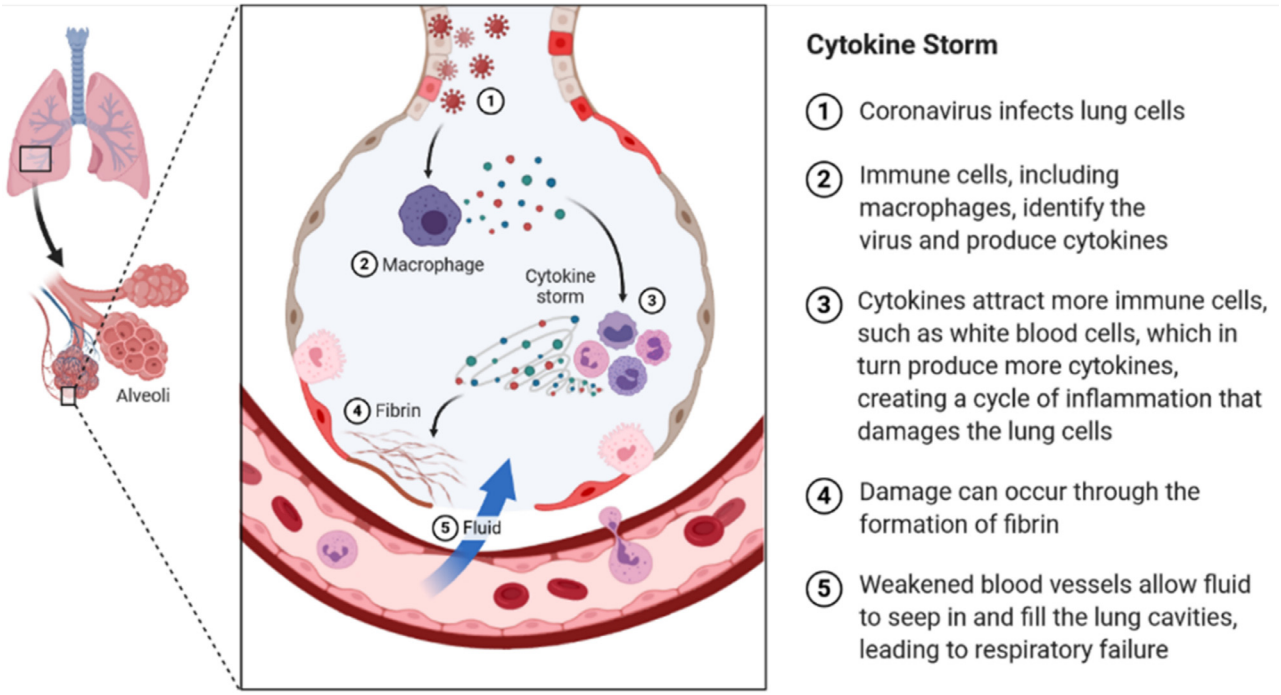

Fig. 1. Cytokine storm induced by coronavirus infection.

SARS-CoV-2, the virus causing COVID-19, has a single-stranded RNA genome of approximately 34 kg bases and a nucleocapsid of helical symmetry [15]. EV surface proteins can facilitate viral fusion with cellular membranes, as they do in the case of other viruses [15]. Fusion of EVs containing viral components with target cells stimulates immune and interleukin signaling pathways to boost immune responses in infected individuals [15,16]. Immune activator-proteins and cytokines such as IL-6, IL-1β, and leukotriene-synthesis enzymes are known to neutralize viral contents [16–18]. Therefore, EVs containing viral proteins can serve as a new technological platform for combating viral infection, and may even improve antiviral immune responses [18].

Innovative engineering of EVs for boosting an antiviral immune reaction may revolutionize the development of COVID-19 vaccines [18–20]. However, to achieve these milestones, several considerations regarding safety, ethics, specificity, and toxicity of this EV-based approach will have to be carefully addressed.

Both EVs and coronaviruses communicate via receptors of CD9 and ACE2, which make EVs an ideal candidate for the treatment of the COVID-19 viral infection [20,21]. EV-based vaccines could potentially attenuate inflammation and repair lung cells damaged by SARS-CoV-2 [21,22]. The S protein of the SARS-CoV-2 binds to the ACE2 receptor and facilitates virus entry into the human lung cells [22,23]. Upon entry into host cells, virus RNA is translated into viral components in order to produce more viral particles; the newly-produced viruses could be packaged in EVs and released from infected cells [23,24]. The Coronavirus family of viruses are enveloped, positive-stranded RNA viruses that produce symptoms of fever, cough, fatigue, shortness of breath, and loss of smell and taste in the host [25,26]. In severe cases, it appears that COVID-19 predominantly affects the lower lung cells and induces a cytokine storm in patients, which leads to organ failure and fatal blood clots [26,27]. Moreover, the cytokine storm promotes the acceleration of viral entry and immune evasion [27,28]. SARS-CoV-2 has been found to induce the infiltration of inflammatory lung fluid into the alveolar space, exacerbating alveolar injury and damage, ultimately causing pneumonia [28–30]. Coronaviruses stimulate the production of IL-6 and IL-1b which induce inflammatory programs in immune cells to suppress *T* cell response and initiate cascades of cytokine storm [29,30]. The process by which cytokine storm is induced by SARS-CoV-2 infection is illustrated in Figs. 1 and 2.

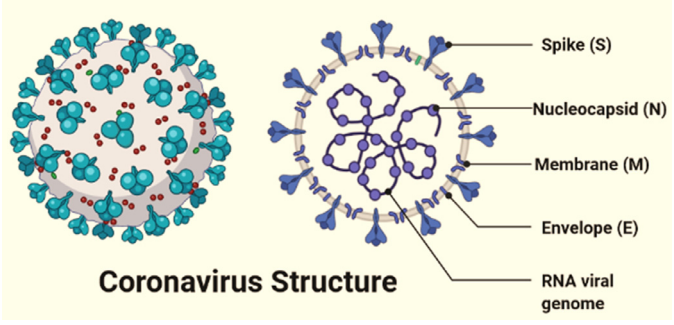

Fig. 2. Stuctural proteins of COVID-19 VIRUS.

Extracellular vesicles and vaccine development

The immunomodulatory features possessed by EVs make EVs a particularly attractive candidate for vaccine development [30,31]. Many studies have shown that EVs can be used for anti-tumor immunotherapy and as therapeutic delivery vehicles in carcinogenesis and infectious diseases [31,32]. Studies have shown that exosomes (a type of EV) carrying bacterial antigens isolated from Mycobacterium bovis and tuberculosis-infected macrophages could generate memory CD4(+) and CD8(+) T cells in the presence of dendritic cells [33,34]. Thus, exosomes or EVs could serve as a promising alternative vaccination solution for SARS-CoV-2 infection [34–36].

Stem cells-derived extracellular vesicles are used for pneumonia-targeted delivery and can improve the therapeutic efficacy while reducing side effects [32,37]. COVID-19 patients derived-EVs suggested that targeted drug delivery to treat pneumonia significantly reduced the cy-

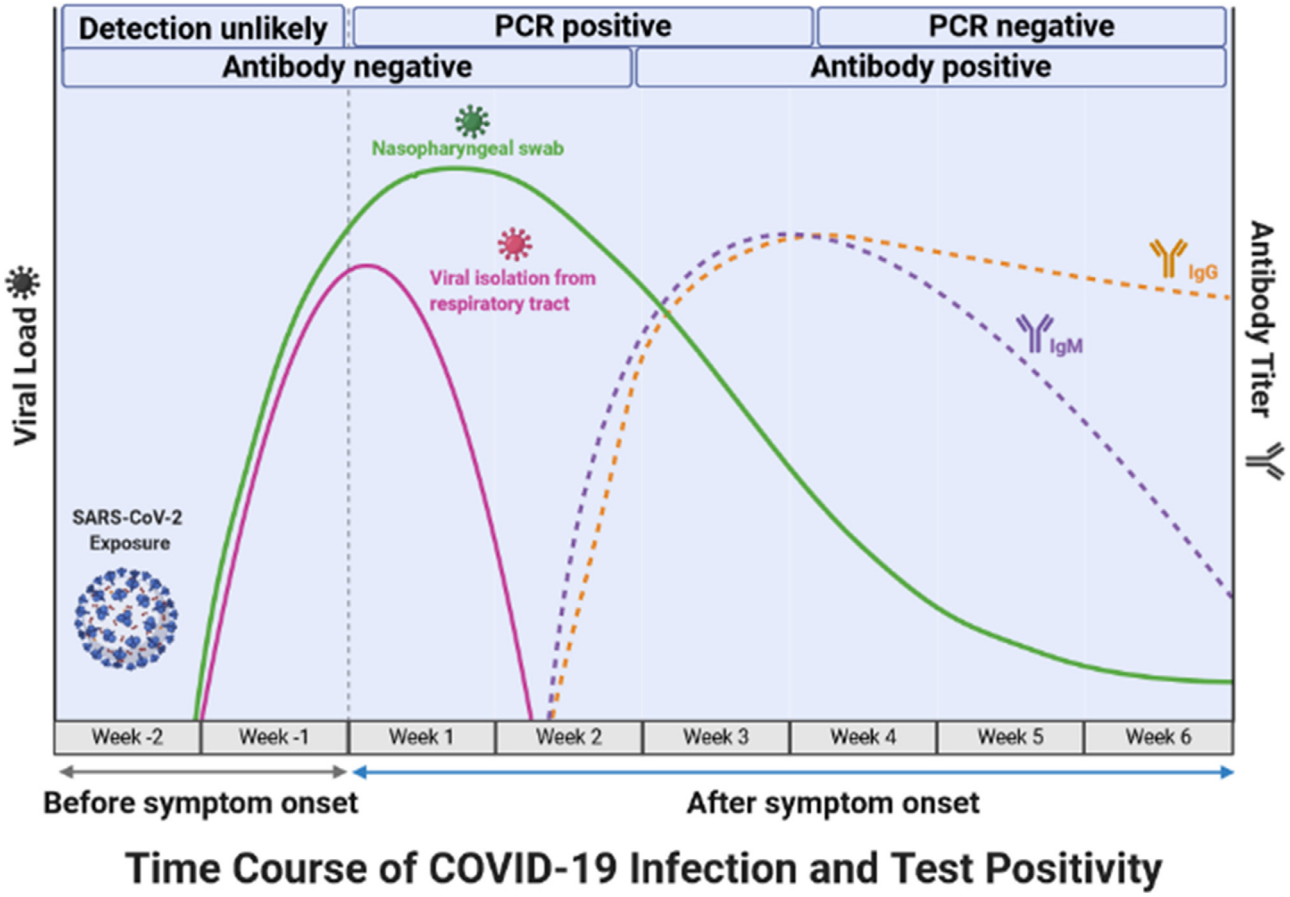

Fig. 3. Time course and antibody devlopment of COVID-19 VIRUS.

tokine storm and inflammation when compared to controls [32,37,38]. These EVs are capable of effectively inducing TH1 cellular response, giving more protection; more so than *B*-cell immunity [38–40]. The EV-induced TH1 response confers protection against airway challenge in mice models [37,41]. In one study, SARS-CoV-2–induced EVs are suggested to be a potential vaccine solution for viral infections [42,43]. Published reports indicate that EVs derived from stem cells (after being transfected by DNA vectors that express Nef mutant/Influenza virus A-NP) could induce cytotoxic T cell response potent enough to kill large antigenic peptide load in mice models, making exosomes a suitable alternate vaccination solution [44,45]. Time course and antibody development of coronavirus is shown in Fig. 3.

Extracellular vesicles and vaccine development for coronavirus

Studies suggest that it is the structural proteins of coronaviruses such as SARS-CoV-2 that allows it to be highly infective and contagious [46–48]. The RNA genome of SARS-CoV-2 has 29,811 nucleotides, encoding for 29 proteins. SARS-CoV-2 has four structural proteins, named E and M proteins, which form the viral envelope; the *N* protein, which binds to the virus's RNA genome; and the *S* protein, which binds to human receptors [35,48,49]. The non-structural proteins include the main protease (Nsp5) and RNA polymerase (Nsp12) [35,49]. Many viruses require surface or spike (*S*) protein proteins for cell fusion and entry [49]. The *S* protein is responsible for host infection and pathogenesis [49,50]. ACE2 is an endogenous membrane protein that facilitates entry of SARS-CoV-2 into host cells and ACE2 is indeed expressed in the lungs of people with lung diseases [50,51]. Studying these different components of the virus, and the role of structural biology in cellular communication with our cells, can offer impressive clues for future therapies [51,52]. The structural composition of coronavirus is shown in Fig. 2.

Angiotensin-converting enzyme (ACE2) is attached to the cell membrane and functions to convert Angiotensin-I and Angotensin-II into other isoforms [22,52,53]. Inhibition of ACE2 has been shown to reduce SARS-CoV-2 infection [53,54]. Because the ACE2 receptor plays a key role in the entry point for SARS-CoV-2 into cells [54–56], directly targeting the ACE2 receptor and Ang II level may provide new therapeutic options for treatment of coronavirus [56]. High levels of Ang II might cause increased pulmonary inflammation and associated lung pathology [56,57]. Understanding the role of ACE2, and the use of small extracellular vesicles to deliver ACE2 to targeted tissues, may even prevent future outbreaks of coronaviruses [57,58].

The SARS-CoV-2 is an enveloped, positively stranded RNA virus. SARS-CoV uses spike glycoprotien (*S*) to interact with DC-SIGN family, and ACE2 gains access into the target cells [59,60]. This makes the *S* glycoprotein a key target for vaccine development [60]. As part of vaccine development, *S* glycoprotein is incorporated into EVs by replacing transmembrane and cytoplasmic domains by VSV-G (G protein of vesicular stomatitis virus) [60–63]. The resulting vaccine consisting of EVs with S-glycoprotein (SGTM) [7,63,64]. Furthermore, they compared the immunogenicity of S glycoprotein–containing EVs with the adeno viral vector vaccine (Ad-SGTM) [64–66]. Both of these vaccines could successfully induce neutralizing antibodies [66]. Further study shows that mice primed with S-glycoprotein containing EV vaccines and boosted with adeno viral vector vaccine (SGTM + Ad-SGTM) produced relatively efficient antibody response when compared to the convalescent serum of the SARS patient [66,67]. This study suggests the potential of utilizing EVs in the development of the COVID19 vaccine. Viral entry and immune response of coronavirus in lung epithelial cells is shown in Fig. 4.

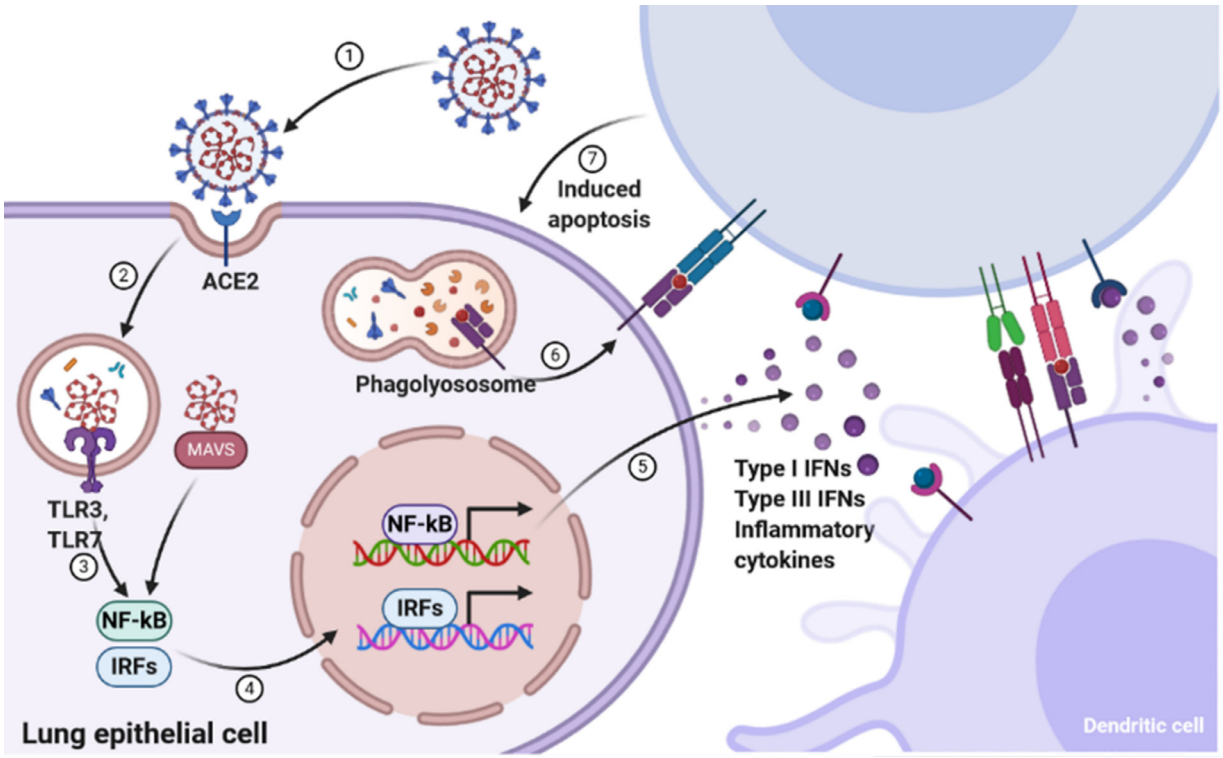

Fig. 4. Viral entry and immune response of cornavirus in lung epithelial cells.

Extracellular vesicles and tissue repair

In the human body, tissue repair activities are carried out by various types of stromal and progenitor cells [68,69]. EVs released by these stromal cells regulate many key biological functions, such as immunomodulatory activities, tissue regeneration processes, and their involvement in spreading viral infections [70,71]. EVs are therapeutic agents that are able to stimulate the regulation of tissue repair and remodeling activities [72]. The EV activates the recipient cells by inducing a cascade of signaling pathways that initiates cellular repair process [73–75]. Tissue repair occurs through the various steps of apoptosis inhibition, inflammation, angiogenesis, and fibrosis [75]. Previous studies have shown that dissolution of this EV-lipid bilayer by proteinase *K* and trypsin led to deactivation of downstream events, therefore reducing the regenerative-properties of EVs [75,76].

Apoptotic inhibition is the initial step in tissue repair [76,77]. Studies have shown that, as exemplified by myocardial injury caused by myocardial ischemia, exogenously administered mesenchymal stem cell and cardiac progenitor cell–derived EVs produce cardioprotective factors which could reduce scar formation and improve cardiac function [77–79]. Similarly, apoptotic inhibition by over-expression of stromal-derived factor 1 and macrophage migration inhibitory factor are crucial for tissue repair [80]. It is evident that heat shock proteins secreted by EVs have antiapoptotic and antioxidative functions and promotes tissue regeneration [80–82].

EVs illicit several responses such as the suppression of CD4+ and CD8+ cells, induction of regulatory *T* cell proliferation by impairing dendritic cell maturation to carry out these immunomodulatory activities, and tissue regeneration [83,84]. Expression of CD73 on the surface of mesenchymal stromal cell–derived EVs is one method used by cancer cells and *T* regulatory cells to suppress immune responses [83–85]. TLR-MyD88-NF-kB is one of the signaling pathways for anti-inflammatory cytokine production and immunosuppression [84,86–88]. EV proteins are known to be one of the potent activators of this TLR-MyD88-NF-kB pathway [87–89].

Vascular endothelial growth factor (VEGF), encapsulated in EVs, play a critical role in inducing the intracellular signaling pathways that lead to angiogenesis [90,91]. EVs expressing DMBT1 and stromal-derived factor 1 as their surface proteins are potent inducers of VEGF, thereby promoting tissue repair [90,91]. Other examples, like *T* cell-EVs, express Sonic Hedgehog proteins, which induce vascular protection through canonical hedgehog signaling [92,93]. EVs carry several proteins, such as hepatic growth factor and sphingosine kinase 2, that are helpful in promoting cellular proliferation and migration, where these EVs could be released by adjacent healthy cells [93,94]. Similarly, morphogens like Wnt ligands participate in inducing intracellular signaling pathways that lead to proliferation of skin cells and wound repair [94,95].

Extracellular vesicles and immune response

Increasing evidence shows that EVs play a very important role in modulating host innate immune responses [75,96]. Innate immune system is the acute and non-specific defense mechanism which serve as the first line of defense against pathogens in the human body [75,96,97]. They are composed of neutrophils, monocytes, macrophages, dendritic cells, natural killer cells and antigen presenting cells [96,98]. EVs such as exosomes derived from host cells (both immune and non-immune cells), tumor cells or from bacteria or viruses mediate immune responses by way of transferring antigens and activation of CD4+, CD8+ *T* cells directly or indirectly through antigen presenting cells [8,99,100]. EVs produce direct activation of CD4+, CD8+ *T* cells without mediation of peptide-MHC complex through a mechanism known as cross-dressing [100,101].

Exosomes with their immunomodulatory capacity, play a dual role [101]. They can activate as well as suppress the immune system depending on the circumstances [102]. For example, the exosomes released from intestinal epithelial cells have global immunosuppression capacity [103]. A recent study shows the immunomodulatory effects of EVs that are derived from retinal pigment epithelium (RPE) [104–106]. Age re-

lated macular degeneration (AMD) is an irreversible disease of the eye [104–106]. In AMD, there will be irreversible degeneration of the retinal pigment epithelium (RPE) [105–107]. RPE is a crucial part of the eye, which maintains the integrity of the blood-retinal barrier [107]. One of the important functions of the RPE is protecting the eye from inflammatory processes, but in AMD, the RPE undergoes irreversible damage in an hyper-inflammed environment [106,108].In one of the vivo studies, scientists created one artificial inflammatory environment and studied exosomes that are released from cytokine induced RPE cells [106,108]. They compared the immunoregulatory effects of exosomes released from stimulated and unstimulated RPE, both of these showed a decrease in *T* cell proliferation [108]. Exosomes from unstimulated RPE made a shift in the monocyte population from CD14, CD16 to CD14++, CD16+ which suggest an increase in the immunoregulatory functions in the monocytes [104,109]. At the same time, exosomes from stimulated RPE could induce monocyte death [109,110].These observations on RPE derived exosomes were further strengthened by more studies showing similar results on *T* cells [110].

Many therapeutic strategies exploiting the immunomodulatory properties of EVs are currently in development [37,111]. Exosomes from immune cells are strongly immunogenic [37,111] and have a low side effect profile [37,112]. Collectively, these properties make EVs effective drug carriers [112]. Clinical trials are in progress to test the efficacy of EVs carrying anti-tumor drugs in treating some cancers [108]. These EVs are developed as 'anti-tumor vaccines' to target patients with early stages of cancer [108].

Interferon and Natural Killer (NK) cells are parts of the innate immune system [113]. They play a central role in killing virus infected and malignant cells [113,114]. Exosomes derived from NK cells are being tested for their utilization in aggressive melanoma [114]. Dendritic cell-derived exosomes are shown to induce NK cell function [114,115] in non-small cell lung carcinoma [115]. In summary, engineered and modified exosomes hold great promise in the development of anti-tumor vaccines and therapeutics [116].

Extracellular vesicles and inflammation

EVs play a crucial role in regulating inflammation [117]. They are useful therapeutic vehicles in the treatment and prevention of inflammatory diseases as well as other immunoregulatory conditions [117,118]. EVs activate damage-associated molecular patterns, cytokines, autoantigens and tissue-degrading enzymes to attract the immune cells towards site of damage or infection [74,118]. For example, EVs are released from ischemic or diseased tissues are implicated in the initiation of inflammation and in the modulation of immune response [118]. EVs released by damaged tissues and have a prominent role in the regulation of inflammation [119]. Inflammatory conditions, such as infections and autoimmune diseases, communicate with other immune cells through EVs for tissue repair and regeneration [119,120]. The biological cargo carried by EVs such as miRNAs, proteins, growth factors, and cytokines play key role in cellular communication during inflammatory and immune responses to lung injury [120]. To combat inflammation, EVs release their cargo to the site of damage and attract other immune cells to accelerate the process of tissue regeneration [120,121]. The field of EVs research is still in an early stage, but has already proven to have profound therapeutic potential in treating dysregulated inflammation, which is a common pathological condition in many diseases [120,122]. The treatment of inflammation is currently gaining much attention as it is a crucial step in various diseases [120]. For inflammation targeting and modulation, danger-associated molecular patterns, activate the inflammasomes in immune cells, amplifying inflammation [120]. Inflammasome-induced activation of EVs then calm down cytokine and stimulate the tissue repair process [123]. The therapeutic utility of EVs for the regulation of immune responses and inflammation show enormous importance in the field of drug development.

Future directions

Artificial intelligence–based or technologically based design and engineering of EVs could be tapped on to prevent viral infection or to enhance antiviral activity towards SARS-CoV-2 infection [122,124]. The effective use of big data sciences, new technologies, and artificial intelligence would accelerate the process of obtaining viral preparations with enhanced biological activity [125]. To do this, certain open questions should be answered, such as how to efficiently obtain EVs with specific (viral) surface proteins that is able to target lung cells and how to scale up the production of EV-based vaccines so that we have sufficient quantities for clinical trials and future use.

Leveraging on the power of advanced technologies and the rapid progress of artificial intelligence can accelerate the clinical trials of COVID-19 vaccines [124,126] with the goal of boosting immunogenicity and protective immunity in patients [126,127]. Vaccination is an effective way to prevent coronavirus infection, halt its transmission, and develop herd immunity [58]. Artificial intelligence has improved diagnosis and treatment outcomes in cancer patients and can be used for clinical diagnosis of the COVID-19 [58,122]. EV-based technology is one such emerging approach to combating viral infections [125]. Recently, the production of EV-conjugated SARS-CoV-2 has shown impressive progress, accelerating the race to develop new therapeutic options [128]. EVs are currently tested in various clinical trials, while other antiviral vaccines and drugs are also planned to be developed for testing in clinical trials [129].

Author's contributions

AU, MU: Conceptualization, data mining, coordinated the project and wrote the paper, AU, MU, Abdullah, GR, LK: Data analysis and writing of review manuscript. AU, MU, SJ: Conceptualization, manuscript review, editing suggestions, and final approval. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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