



Targeting the PANoptosome with miRNA Loaded Mesenchymal Stem Cell Derived Extracellular Vesicles; a New Path to Fight Against the Covid-19?

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Coronavirus disease 19 (Covid-19), as announced on March 11, 2020, is a pandemic caused by the SARS-CoV-2 virus that affects the whole world and can cause fatal consequences [1]. Common clinical findings of Covid-19 includes fever, dry cough, fatigue, headache, dizziness, abdominal pain, nausea, vomiting, anosmia, dysgeusia, and diarrhea. Depending on the severity of the disease, clinical signs of coagulation defects, cardiac dysfunction, liver injury, renal dysfunction may develop. Reductions in total lymphocytes, CD4 + and CD8 + T-cells, B-cells, and natural killer cells, elevations in D-dimer levels, C-reactive protein (CRP), lactate dehydrogenase (LDH), and high-sensitivity cardiac troponin I and ground-glass opacities in computed tomography are laboratory findings of the disease [2]. Inappropriate immune system activation and cytokine storm can also be seen in the later stages of the disease [3]. It is known that different viruses, fungi and bacteria activate inflammatory cell death pathways so far [4]. PANoptosis is a new concept that has emerged recently and refers to the combined activation of inflammation-mediated pyroptosis, apoptosis and necroptosis-type cell death processes through PANoptosome [5, 6]. This cellular process may explain the link between tissue damage, reduced number of immune cells and inappropriate immune system activation seen in Covid-19. PANoptosome consists of Caspases 1/6/8, NLR Family Pyrin Domain Containing 3 (NLRP3), Apoptosis-Associated Speck-Like Protein Containing A CARD (ASC), Receptor Interacting Serine/Threonine Kinase 1/3 (RIPK1/3) and Z-DNA Binding Protein 1 (ZBP1) proteins [7]. In the inflammasome induced PANoptosis process, pyroptosis,

apoptosis and necroptosis is executed through Gasdermin D (GSDMD), Caspases 3/6/7 and Mixed Lineage Kinase Domain-Like Protein (MLKL) proteins, respectively [8]. Although Lee et al. stated that SARS-CoV-2 infection does not activate necroptosis arm of the PANoptosis there are also publications suggesting the opposite [9]. The study conducted by Zheng et al., and observed that PANoptosis is induced by NLRP3 in bone marrow-derived macrophages infected with Murine Hepatitis Virus, which is murine corona virus, shows that corona viruses can induce PANoptosis [10]. Also in another report it was stated that cytokines TNF- α and IFN- γ , which are related to SARS-CoV-2 infection, stimulated the pyroptosis, apoptosis, and necroptosis pathways with a synergistic effect in mouse bone marrow derived macrophages while blokage of Signal Transducer And Activator Of Transcription 1 (STAT1) / Interferon Regulatory Factor 1 α (IRF1) pathway suppressed PANoptosis [11].

In some studies on Covid-19 patients, it was determined that cell death pathways are activated in different types of cells. It is known that apoptosis is increased in B and T-cells in the peripheral blood of Covid-19 patients and it is associated with the disease severity [12, 13]. Li et al., reported that, in the lung biopsy materials obtained from two patients apoptosis and pyroptosis increased in type 1 and 2 pneumocyte and endothelial cells [14]. It has also been shown that inflammation-mediated pyroptosis is activated in monocytes infected with SARS-CoV-2 and in monocytes of Covid-19 patients [15]. Also in some reports it was reported that, PANoptosome components were overexpressed or activated in cells of Covid-19 patients. In a report it was suggested that the level of RIPK3 in the peripheral blood of patients with a severe course of Covid-19 was higher compared to patients with a mild course [16]. One of the components of the PANoptosome complex is NLRP3. Analysis of SARS-Cov-2 infected Calu-3 lung epithelial cells and lung tissue of patients who died due to Covid-19 showed that apoptotic and necroptotic pathways were activated by NLRP3 and Caspase 8 [17]. Studies conducted in peripheral blood mononuclear

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cells of Covid-19 patients and in tissue samples taken from patients who died due to Covid-19 have shown that NLRP3 inflammasomes are activated [18, 19]. Beyond that, there are publications stating that one of the mechanisms of action of chloroquine and hydroxychloroquine drugs might be to suppress the NLRP3 inflammasome [20]. However, it should be kept in mind that the clinical studies conducted show that the use of hydroxychloroquine in patients hospitalized due to Covid-19-induced lung distress has no superiority over placebo [21]. In addition, it is another important issue that the use of chloroquine and hydroxychloroquine has been shown to cause cardiac toxicity in Covid-19 patients [22]. Proteomic analysis performed on the plasma of Covid-19 patients showed that Caspase 8, which is a component of the PANoptosome, is upregulated compared to normal individuals [23]. One of the important components of the PANoptosome complex is RIPK1. It was determined that Covid-19 patients had active and phosphorylated RIPK1 in their respiratory tract epithelium biopsies [24]. Also, bioinformatics analyzes have determined that RIPK1 is an important hub gene that plays a pivotal role in SARS-CoV-2 viral pathogenesis in many tissues [25].

Today, we know that there are clinical studies on the usability of mesenchymal stem cell-derived extracellular vesicles in the treatment of Covid-19 [26]. miRNAs are 20–22 nucleotide long single stranded RNA molecules that play important roles in regulating gene expression. The publication of Schultz et al., (2021) which was as one of the pioneering works on this subject. In this publication, the researchers stated that some miRNAs found in extracellular vesicles (EVs) originating from mesenchymal stem cells have the ability to bind to mRNAs from which proteins in the PANoptosome complex are synthesized, thereby suppressing protein synthesis. In this study, the researchers examined miRNAs common in at least two data sets in a total of five EV gene expression datasets isolated from umbilical cord (1), bone marrow (3), and adipose-derived mesenchymal stem cells (1). They determined a total of 266 common miRNAs in these EVs. When they examined the interaction of these miRNAs with the mRNAs of these PANoptosis genes by bioinformatics analysis, they determined that some of these miRNAs have the capacity to bind to the 3'UTR regions of the mRNAs of PANoptosis genes. However, when these miRNAs are examined in detail, it is seen that there is no miRNA that collectively blocks the pathways of pyroptosis, apoptosis and necroptosis. [27]. These reports provide hints that PANoptosis may be one of the factors that play a role in Covid-19 pathogenesis. More studies are needed within the scope of PANoptosis to confirm its accuracy. In a recent review, the preclinical applications of miRNA loaded bone marrow derived mesenchymal stem cell derived EVs in different disease models were discussed [28]. If PANoptosis is an important process in Covid-19 pathogenesis, it may be possible

to suppress this process with different miRNA molecules that reduce the expression of components of this pathway. These miRNAs can be transferred to target cells using mesenchymal stem cell-derived vesicles as nanocarriers. In this system, since multiple miRNAs can be loaded into the same EVs, suppression of all three PANoptosis related pathways can be accomplished. The miRNA molecules loaded on these EVs can bind to the mRNAs of the proteins involved in the PANoptosis complex in target cells, thereby reducing their expression. With this approach, the efficiency of mesenchymal stem cell derived extracellular vesicles can be improved.

In summary, these and similar studies on PANoptosis, which is a very current issue, are important for innovative new treatment approaches to be developed for fight against Covid-19 disease.

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Declarations

Consent for Publication All authors of the manuscript; have read and agreed the Journal BioMed Central 'Copyright and License Policy', have read and agreed to its content and are accountable for all aspects of the accuracy and integrity of the manuscript in accordance with ICMJE criteria and declare that 'The Article' is original, has not already been published in a journal, and is not currently under consideration by another journal.

Conflict of Interest This manuscript is only prepared for scientific purposes and 'There is no conflict of interests'.

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