Heliyon 10 (2024) e39303

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Alterations in the expression of serum-derived exosome-enclosed inflammatory microRNAs in Covid-19 patients

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ARTICLE INFO

Keywords:
COVID-19
Extracellular Vesicles
miRNA-21
miRNA-218
miRNA-155
miRNA-10b

ABSTRACT

Introduction: MicroRNAs in exosomes play a role in biological processes such as inflammation and Epithelial-mesenchymal transition (EMT). In EMT, epithelial cells undergo phenotypic changes and become similar to mesenchymal cells. EMT increases the invasion and metastasis of cancer cells. We aimed to evaluate the expression levels of miRNA-21, miRNA-218, miRNA-155, and miRNA-10b, which are effective in the pathway of inflammation and EMT in serum-derived exosome of COVID-19 patients.

Method: Blood samples were taken from 30 patients with COVID-19 and five healthy individuals as a control group. After separating the serum from the collected blood, the exosomes were purified from the serum. Relative expression of microRNAs was measured by real-time PCR method.

Results: The relative expression of miRNA-21, miRNA-218, and miRNA-155 in serum-derived exosomes of patients with COVID-19 had a significant increase ($p < 0.0001$). Also, the relative expression of miRNA-10b was significantly increased in the patient group ($p < 0.01$), but the changes in the expression level of miRNA-10b were not as significant as the changes in the expression level of other microRNAs.

Conclusion: miRNA-21, miRNA-218, miRNA-155, and miRNA-10b are involved in the pathogenesis of COVID-19 disease, and their transmission by exosomes leads to pathogenic lesions and problems in other parts of the body.

1. Introduction

Coronavirus disease-2019 (COVID-19) is a critical global health problem that has claimed the lives of thousands of people worldwide [1]. The causative agent of the pandemic is severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) [2]. It generally has two clinical consequences. In the first consequence, SARS-CoV-2 infection leads to the destruction and lysis of its target cells. It

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https://doi.org/10.1016/j.heliyon.2024.e39303
Received 11 June 2024; Received in revised form 5 October 2024; Accepted 11 October 2024
Available online 12 October 2024

leads to complications such as a respiratory infection similar to the flu virus. In the second consequence, the disease enters a severe inflammatory phase. Under this consequence, the expression of inflammatory genes and cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-1 (IL-1) increases, and the disease progresses to acute respiratory distress syndrome (ARDS). At this stage, there is not much hope for the patient to recover [3,4]. In this regard, various studies showed a direct and significant relationship between its severity and the expression of inflammatory genes [5–9]. Virus-infected cells can transmit inflammatory mediators and regulators of inflammation to the adjacent cells by secreting exosomes, thereby causing involvement in other cells. Exosomes are a type of cellular transport system that cells use to communicate [10]. Exosomes are vesicles in the size of 30–200 nm secreted from cells under pathological and physiological conditions [11,12].

Exosomes contain microRNAs that can regulate their target gene expression by identifying and binding to 3'-untranslated regions (3′-UTR) [13]. MicroRNAs are short non-coding RNAs, usually composed of 10–15 nucleotides. This group of RNAs plays a role in fundamental biological processes such as differentiation, proliferation, apoptosis, inflammation, and immune system regulation [14–16].

Recently, the role of microRNAs as essential regulators of the human immune system in lung disease, respiratory distress syndrome, and respiratory tract inflammation has been identified. Also, in viral diseases, it has been revealed that host microRNAs can prevent virus replication in the host cell by directly destroying the RNA-infected virus genome or suppressing the translation of viral proteins [17,18]. Besides, microRNAs are important host regulators in generating inflammatory responses to prevent the spread of viral disease [19]. In this regard, research has shown the association of several specific types of microRNAs with the immune responses against various respiratory viruses, including respiratory syncytial virus (RSV), influenza virus (IV), human rhinovirus (hRV), and human Coronavirus (HCoV) [20,21]. The human Coronavirus OC 43 (HCoV-OC43) can bind to microRNA-9, a negative regulator of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and silence this microRNA, and finally indirectly activate NF-κB [20]. Also, the virus has been shown to select specific microRNAs from the host to disrupt the antiviral immune response. The Coronavirus Transmissible Gastroenteritis Virus downregulates microRNA-30a-5p expression, thereby disrupting the type 1 interferon response, suppressing the defense responses against itself in this pathway [22].

Epithelial-mesenchymal transition (EMT) is a unique process affected by various microRNAs. EMT is a biological process in which epithelial cells lose their phenotypic properties and regain the phenotypic properties of mesenchymal cells. It is commonly seen in the process of growth, wound healing, and tissue fibrosis. Unfortunately, this process can be used by cancer cells, which often leads to tissue invasion, cancer cells' resistance to apoptosis, and failure to treat cancer [23].

Evaluation of the expression level of effective microRNAs in the EMT pathway, which has the potential for carcinogenicity and metastasis in cancer patients, is also of considerable importance in patients with COVID-19. Many microRNAs, such as microRNA-21 and microRNA-10b, are involved in the EMT process and promote metastasis in cancer patients [24,25]. Also, microRNAs, such as microRNA-21, microRNA-10b, microRNA-218, and microRNA-155, are involved in inflammatory pathways, and their expression levels increase during inflammation [26–33].

The study of the expression of microRNAs in the path of EMT and inflammation is of considerable importance. As it uncovers biological processes taking place in the cell, understanding these processes helps to design drugs that treat viral infections effectively. Also, the expression of specific microRNA profiles during infection can be used as a faster and better approach to diagnosis and monitoring of the disease [34,35].

Based on our research, no study has been performed to investigate the expression level of microRNAs involved in inflammation and EMT in serum-derived exosomes of patients with COVID19. This study aimed to evaluate the expression levels of miRNA-21, miRNA-218, miRNA-155, and miRNA-10b, which are effective in the pathway of inflammation and EMT in serum-derived exosomes of patients with COVID-19.

2. Materials & methods

2.1. Samples

In the present study, blood samples were taken from 30 patients with mild to moderate COVID-19 whose condition was confirmed by real-time PCR, and Five healthy individuals (Age range from 30 to 65 years 2 women and 3 men) as controls who had no previous infection or inflammatory disease. Notably, patients with COVID-19 had not been treated with any medication before blood sampling. The patients participating in this project did not have underlying diseases such as diabetes and hypertension and written informed consent was obtained from all participants in this study. Demographic and clinical information of patients were also collected by studying their files. Blood samples were centrifuged at 4000 rpm for 10 min to separate serum. Also, the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. This research was approved by the ethics committee of Ahvaz Jundishapur University and the ethics code of this study was IR.AJUMS.REC.1399.176.

2.2. Isolation of serum exosomes

The exosomes were purified from 1 mL of serum obtained from COVID-19 patients. Initially, the serum was filtered using a 0.22 μm filter and then centrifuged at 3,000g for 15 min to remove cell debris. Following the instructions of the ExoQuick kit (ExoQuick, System Biosciences LLC, USA), the serum was mixed with Regent A at a ratio of 5 to 1. The mixture was vortexed for 5 min and then left at 4 °C overnight. After 24 h, the serum and Regent A mixture were centrifuged at 4500 rpm for 45 min at 4 °C, and then the exosomes were

extracted.

2.3. Dynamic light scattering and flow cytometric assessment of serum-derived exosomes

The size and homogeneity of the exosomes we extracted were assessed using dynamic light scattering (DLS). Following the instructions of the Particle Size Analyzer (PSA, Scatteroscope-I, Qudix, Korea), the samples were diluted in sterile, particle-free PBS at a ratio of 1:2000 and then mixed or ultrasonicated to ensure uniform distribution of the particles. The diluted samples were then injected into the laser chamber at 25 °C. This process was repeated twice for accuracy. Furthermore, CD63 and CD9 have been identified as specific biomarkers for the assessment of exosomal surface markers. In this study, exosomes that were incubated with latex beads were subjected to staining with anti-CD9 and anti-CD63 antibodies (Abcam, Cambridge, UK), followed by analysis utilizing a FACS caliber flow cytometer (BD Bioscience, San Diego, CA, USA) in conjunction with Flow Jo software (San Jose, CA, USA).

2.4. Scanning Electron Microscope of serum-derived exosomes

The shape and size of exosomes isolated from the serum of patients with COVID-19 were examined using a Scanning Electron Microscope (SEM). First, a 5 μl drop of exosome was diluted in deionized water for 2 min, and the exosome suspension was fixed with 2 % paraformaldehyde. Next, 2 μL of the sample prepared in the previous step were placed on silicon chips and then dried in acetone for 6 min. Finally, images were captured with the SEM at 30 kV after gold-palladium sputtering. The SEM used for this examination was the KYKY-EM 3200 by KYKY Technology Development Ltd, China.

2.5. Bicinchoninic Acid Assay

Exosome protein concentration was measured with BCA Protein Assay Kit (Thermo Scientific Pierce). For this objective, according to the manufacturer's instructions, BSA standards, and samples (25 μl) were set in a 96-well plate, blended with the working solution (200 μl) and incubated for 30 min at 37 °C and later examined by spectrophotometer at 562 nm (MPR4+; Hiperion, Roedermark, Germany).

2.6. Total RNA extraction and cDNA synthesis

To extract the total RNA from the purified exosomes, according to the instruction, miRCURY™ RNA Isolation Kit (Biofluids – Exiqon, USA) was used. Also, the quality and concentration of total RNA extracted were determined by NanoDrop (NanoDrop 2000, Thermo Fisher Scientific, USA). Then cDNA was synthesized from the RNA, according to the miScript II RT kit instruction (Qiagen, USA). Poly-A method was adopted for DNA synthesis. In this method, the poly Adenine sequence is added to all the RNAs in the sample, and then cDNA is synthesized from the polyadenylated RNAs. The synthesized cDNAs were stored at minus 20 °C until further testing.

2.7. Evaluation of microRNA expression level by real time PCR

Real-time PCR was utilized to obtain the expression level of microRNAs. In this experiment, first, the synthesized cDNAs were diluted, and then, using specific primers, the samples were prepared for real-time PCR. Reaction components were comprised of SYBR Green Master Mix (Takara, Japan), two μl diluted cDNA, and specific primers miRNA-21, miRNA-218, miRNA-155, and miRNA-10b as well as water nuclease-free. Also, the U6 gene, a short-sequence RNA, was used as an internal control to normalize the results. Nucleotide sequence of forward primers miR-155-5p, miR-21-5p, miR-218-5p, miR-10b-5p and U6 were, respectively,5′- TTAATGCTAATCGTGATAGGGGTTAA-3′,5′-GCAGTAGCTTATCAGACTGATGT-3′,5′-GGGTTGTGCTTGATCTAACCAT-3′,5′-GTGTTTAAGCCAAGATGTCCCAT-3′ and 5′-CGCAAGGATGACACGCAAATTC-3′. Conversely, the sequence of the common reverse primer that hybridizes to the oligo-dT adapter was 5′-GCGAGCACAGAATTAATACGAC-3'.

After combining the reaction components, the expression of genes was investigated using the desired temperature program. Finally, the Applied Biosystems Step One TM (Life technologies, Waltham, USA) and related software Step one V2.2.2 were used.

2.8. Statistic analysis

For statistical analysis between clinical factors, the normality of the data was first ensured followed by independent sample *t*-test. Mann-Whitney *U* test was also run due to abnormal distribution of WBC clinical factor data.

The relative expression of miRNA-21, miRNA-218, miRNA-155, and miRNA-10b in patients with COVID-19 and controls were determined using comparative CT. First, CT, the Number of cycles required to pass the fluorescent signal from the threshold, was determined by real-time PCR method, and then FOLD CHANGE was determined according to formula $2^{-\Delta\Delta\ Ct}$. After determining the intensity of abnormal data distribution, Mann-Whitney U non-parametric test was used. Graph-pad Prism software (version 8) was also used for statistical analysis.

3. Results

3.1. Characteristics of purified exosomes from serum

The spherical structure and size of exosomes purified from the serum of patients with COVID-19 and the control group were confirmed by electron microscopy. The diameter of the exosomes was also determined using the DLS method. Particle Size Analyzer measurement results indicated that the isolated exosomes were uniform in size, represented by a single peak in the distribution plots. This experiment showed that 90 % of the exosomes purified from the serum of patients and the control group were 71.8 nm in diameter. Furthermore, flow cytometric analysis showed high percentages of CD63 and CD9. Scanning electron microscopy images of purified exosomes showed that the exosomes had a spherical structure and the diameter size of exosomes ranged from 55 to 98 nm. (Fig. 1). Also, the protein content (1 mg/ml) of exosomes isolated from patients' serum was determined by BCA method.

3.2. Clinical information of patients

The study was performed on 30 patients with COVID-19, in which 80 % of the subjects were male, and 20 % were female. The mean age of the subjects was 54.5 years. The mean inflammatory factor C-reactive protein (CRP) in patients with COVID-19 was 59.20 mg/L, the lowest of which was 13.90 mg/L, and the highest was 96.00 mg/L. Also, the mean of another inflammatory factor Erythrocyte Sedimentation Rate (ESR) in patients was 59.97 mm/h with a maximum of 102.00 mm/h and a minimum of 13.00 mm/h. Blood oxygen saturation in COVID-19 patients averaged 90.6 %. Demographic and clinical information of patients is also listed in Table 1.

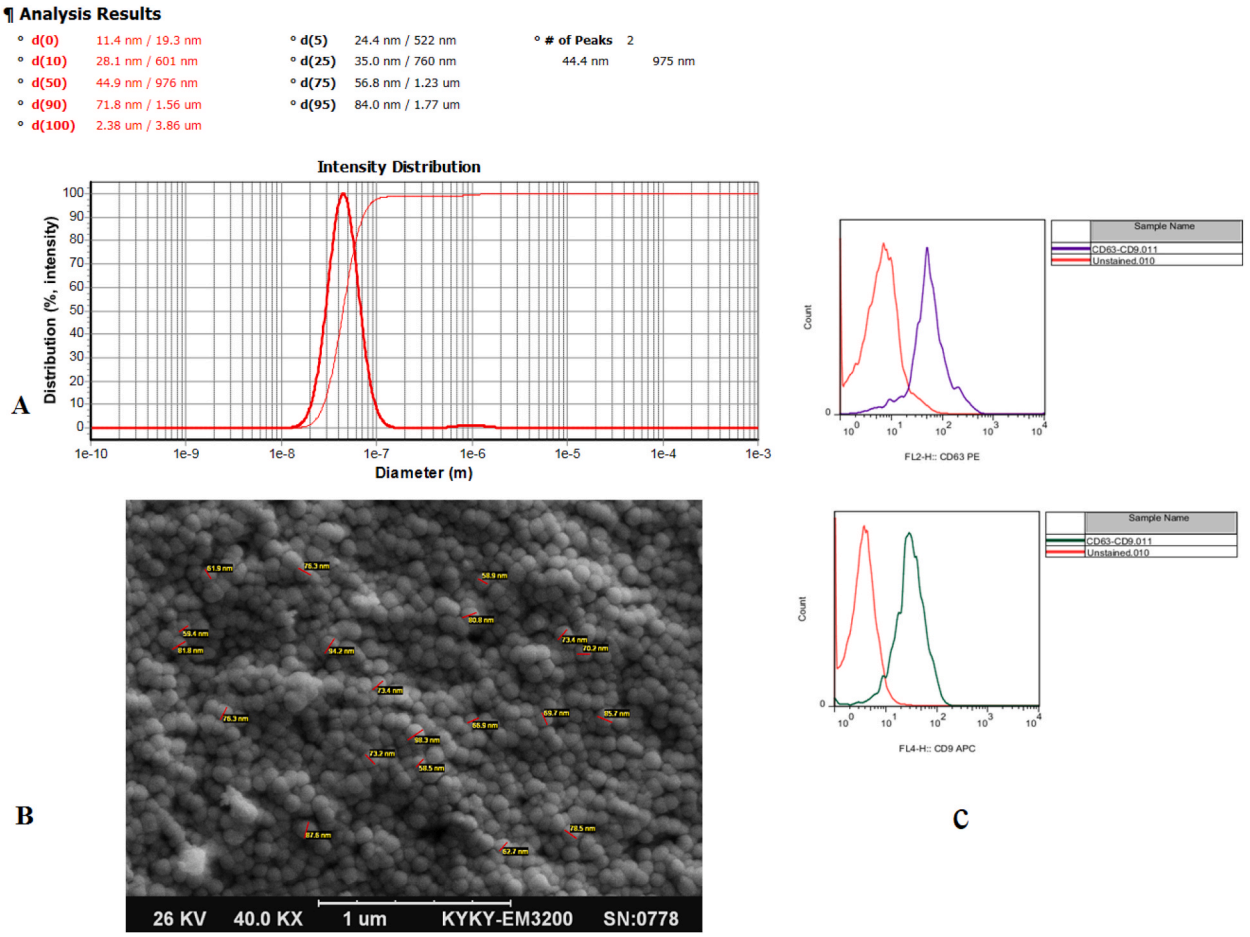

Fig. 1. Characteristics of purified exosomes from serum. (A), DLS test report to check the diameter of the exosomes. (B), Exosomes were imaged and their spherical structure was confirmed by SEM. (c), Flow cytometric analysis of isolated exosomes for surface expression of CD63 and CD9 in the presence of unstained results.

Table 1
Demographic and clinical information of patient.

Code	Age	Gender	Body temperature	Blood pressure	O_2 saturation	WBC (10^3/μL)	Platelets (10^3/μL)	Lymphocyte count (10^3/μL)	Neutrophil count (10^3/μL)	CRP (mg/L)	ESR (mm/hr)
1	67	Man	37.6C	120/80	87 %	4	128	1.13	67.9	37	60
2	72	Man	36C	125/74	77 %	5.2	126	1.43	68.5	36	13
3	76	Man	37C	132/70	88 %	3.2	117	0.52	73.7	13.9	35
4	41	Man	36.6C	115/65	90 %	2.5	138	0.68	65.7	34	50
5	48	Man	36C	140/85	88 %	12.1	181	3.71	63.6	85	85
6	57	Man	37C	110/65	96 %	5.8	159	3.5	68.3	48.4	42
7	60	Female	37.5C	120/80	91 %	17.9	181	2.11	83.3	90	70
8	73	Female	37.5C	110/65	93 %	4.5	182	1.23	68.7	62	30
9	53	Man	38.5C	140/100	94 %	11.6	249	2.05	78	76	85
10	58	Man	37.5C	115/60	94 %	5.6	149	3.5	74.2	52.4	50
11	59	Man	36C	130/80	92 %	9.5	237	1.39	80.5	55	80
12	48	Man	37.4C	120/80	95 %	4.6	234	1.11	70.9	39	44
13	33	Man	37.5C	120/80	87 %	5.9	149	1.08	76.4	81	50
14	69	Female	36.8C	120/70	89 %	4.5	176	0.92	74.3	52.2	80
15	65	Man	36.5C	160/90	89 %	5.2	216	1.09	73.3	80	100
16	51	Man	37.5C	110/80	95 %	5.8	164	2.6	77	56.4	52
17	40	Man	37C	120/80	97 %	6.1	153	0.85	82.2	28	40
18	38	Man	35.5C	110/80	96 %	5.8	135	1.73	65.2	63	55
19	48	Man	35.8C	115/60	94 %	11.4	282	0.79	88.9	60	35
20	39	Female	36.4C	120/60	85 %	11.2	219	1.68	80.3	96	102
21	42	Man	36.5C	120/70	94 %	5.4	131	1.06	77.2	86	60
22	71	Man	37C	120/80	83 %	6.7	220	0.93	84.9	89	90
23	59	Man	37.5C	120/80	94 %	6.2	156	3.5	76.9	57.4	54
24	48	Man	37.2C	170/110	92 %	11.1	174	2.33	74.8	21	15
25	62	Female	38C	140/80	85 %	13.5	195	1.5	63.5	70	82
26	49	Female	37.8C	130/90	90 %	15.8	180	2.1	82.2	69	74
27	56	Man	37.5C	130/80	92 %	16.5	178	2.35	85.3	66	72
28	53	Man	38C	120/80	88 %	14.9	181	3.56	83	62	77
29	50	Man	37.5C	120/80	93 %	16.2	176	2.9	75.9	61	70
30	52	Man	37C	120/80	92 %	6.5	155	1.96	69.9	49.4	47

3.3. Comparison of clinical factors between different groups

In this study, we compared clinical factors including body temperature, oxygen saturation, white blood cell count, CRP, and ESR in the two age groups below the mean age of (54.5) years and above. The statistical analysis results showed that factors including body temperature, oxygen saturation, WBC, CRP, ESR with P-value obtained from statistical analysis were ($p = 0.7468$), ($p = 0.1127$), ($p = 0.3927$), ($p = 0.7414$) and ($p = 0.8035$), respectively and none of the factors mentioned above were significantly different in the two age groups. We also compared body temperature, oxygen saturation, WBC, CRP, and ESR in the two groups with lower and higher blood pressure above (120/80), and the obtained P-values were ($p = 0.9861$), ($p = 0.0865$), ($p = 0.1125$), ($p = 0.7190$) and ($p = 0.4995$), respectively. These P-values indicated no significant difference in any of the factors in the two groups with different blood pressure. Three factors CRP, ESR, and WBC, in two groups with lower and higher oxygen saturation than the average oxygen saturation (90.6 %) were compared. The obtained P-values included ($p = 0.5459$), ($p = 0.2312$) and ($p = 0.6404$). The statistical analysis results demonstrated no significant differences in the factors in the two groups. In addition, regarding the difference between the two factors, CRP and ESR, measured in the two groups with a WBC count lower and higher than the mean ($8.5*10^3$μL), there was a significant difference between the two factors in two groups ($p = 0.0442$; $p = 0.0382$) (Fig. 2).

3.4. Relative expression of microRNAs in both patient and control groups

The results of the experiments showed that the relative expression of miRNA-21 in exosomes derived from patients with COVID-19 significantly increased ($p < 0.0001$). Also, the relative expression of miRNA-218 significantly rose in exosomes derived from them ($p < 0.0001$). Similarly, the relative expression of miRNA-155 in exosomes derived from COVID-19 patients increased significantly ($P < 0.0001$). However, the increase in miRNA-155 expression changes was not as significant as the miRNA-218 and miRNA-21 expression changes.

The relative expression of miRNA-10b was significantly higher in the patient group ($p < 0.01$). However, the expression level rates was not as significant as the changes in the expression level of miRNA-21, miRNA-218, and miRNA-155 (Fig. 3).

3.5. Relative expression of microRNAs in low O_2 saturation group and high O_2 saturation group

The relative expression of microRNAs enclosed in exosomes was compared between the low O_2 saturation group and high O_2 saturation groups to differentiate patients with moderate and mild conditions. The results indicated that the expression of miRNA-21,

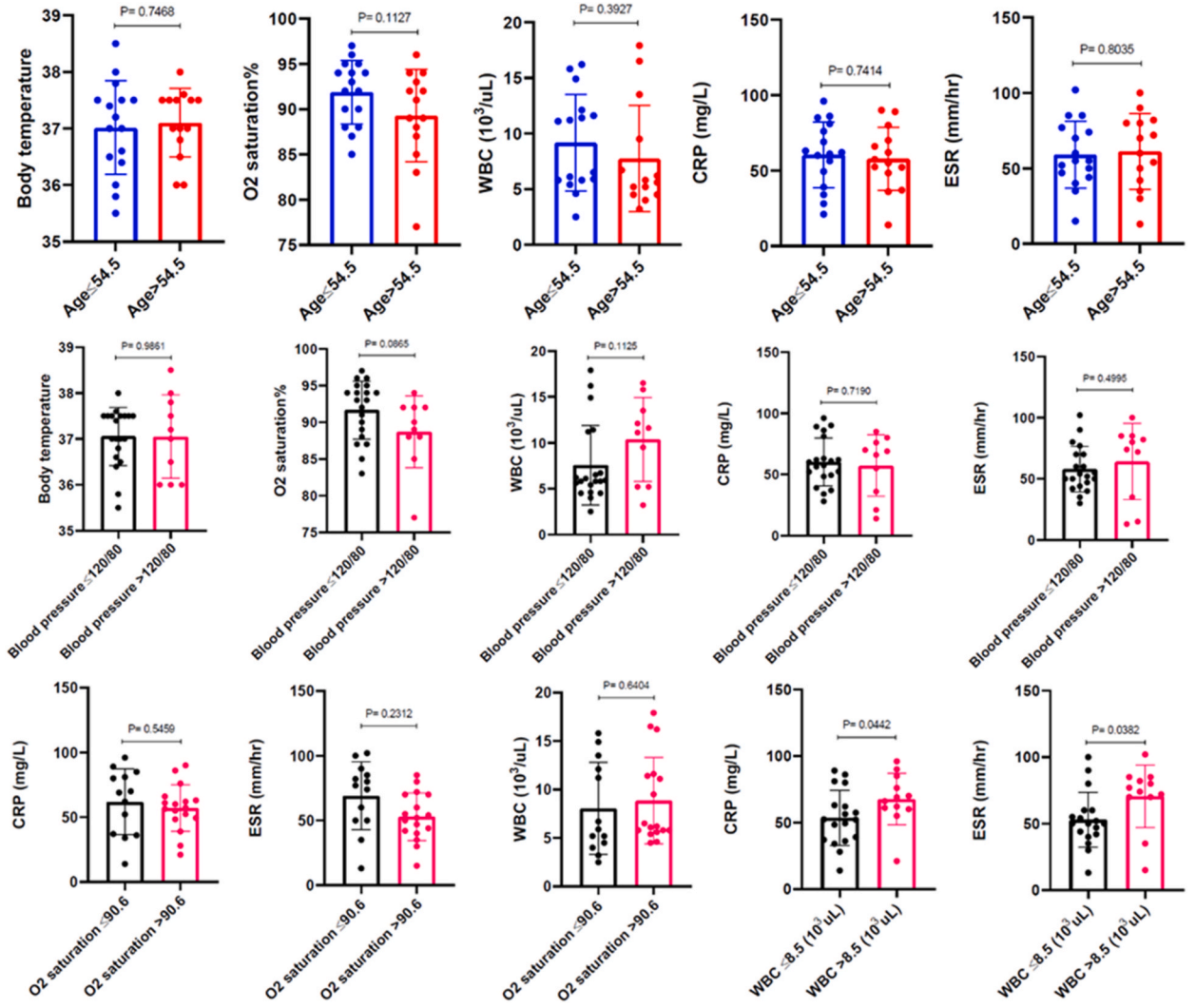

Fig. 2. Comparison of clinical factors including body temperature, oxygen saturation, white blood cell count, CRP, and ESR between different groups by Independent sample *t*-test and Mann-Whitney U non-parametric test. Clinical factors such as body temperature, oxygen saturation, WBC, CRP, and ESR in both (age) groups less and more than 54.5 years were compared and there was no significant difference between the two groups in any of the factors. Factors of body temperature, oxygen saturation, WBC, CRP, and ESR in the two groups with (blood pressure) less and more than 120/80 were compared and there was no significant difference between the two groups in any of the factors. WBC, CRP, and ESR in two groups with (oxygen saturation) less and more than 90.6 % were compared and there was no significant difference between the two groups in any of the factors. CRP and ESR factors in two groups with less (WBC) and more than 8.5* (10^3 μL) was a significant difference.

miRNA-218, and miRNA-155 was significantly higher in the low O_2 saturation group compared to the high O_2 saturation group ($P = 0.0074$, $P = 0.0036$ and $P = 0.04$, respectively). However, the expression of miRNA-10b did not show a significant difference between these two groups ($P = 0.067$) (Fig. 4).

3.6. Correlation between each inflammatory marker (ESR, WBC, CRP) and each microRNA

The results indicate a correlation of ESR with miRNA-21, miRNA-218, miRNA-155, and miRNA-10b ($r = 0.4731$, $P = 0.0083$; $r = 0.6431$, $P = 0.0001$; $r = 0.3844$, $P = 0.036$; and $r = 0.4884$, $P = 0.0062$, respectively). However, the CRP marker did not show any correlation with any of the microRNAs, and the WBC marker was only correlated with miRNA-21 ($r = 0.4077$, $P = 0.0253$) (Fig. 5).

4. Discussion

This study aimed to evaluate the expression level of inflammatory microRNAs in the exosomes of patients with COVID-19. Recently, a correlation has been found between EMT and certain viral infectious diseases. SARS-CoV-2 has been found to trigger

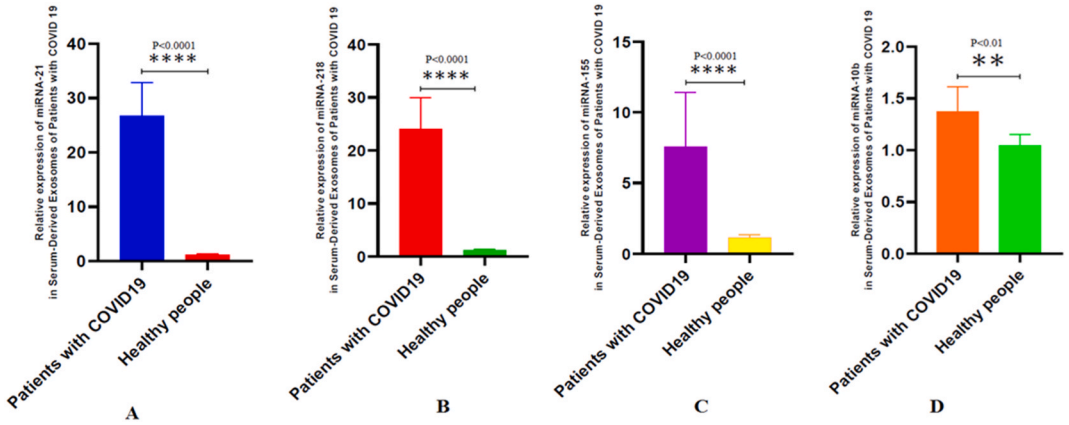

Fig. 3. Relative expression of microRNAs, by Mann-Whitney U non-parametric test (A), the relative expression of miRNA-21 in the patient group was significantly higher than in the control group ($P < 0.0001$). (B), the relative expression of microRNA-218 in the patient group was significantly higher than in the control group ($P < 0.0001$). (C), the relative expression of microRNA-155 in the patient group was significantly higher than that in the control group ($P < 0.0001$). (D), the relative expression of microRNA-10b in the patient group was slightly higher than that in the control group ($P < 0.01$).

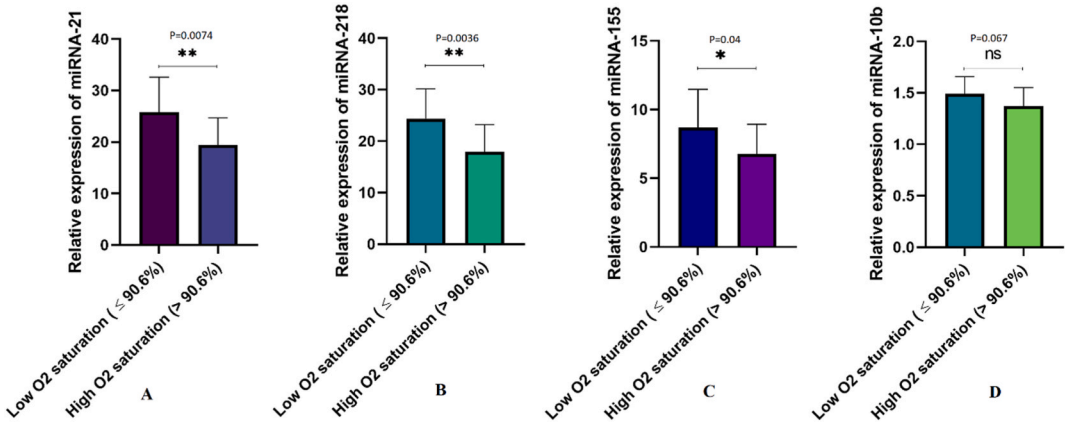

Fig. 4. Relative expression of microRNAs in low O2 saturation group and high O2 saturation group. (A), the relative expression of miRNA-21 in the low O_2 saturation group was significantly higher than in the high O_2 saturation group ($P = 0.0074$). (B), the relative expression of microRNA-218 in the low O_2 saturation group was significantly higher than in the high O_2 saturation group ($P = 0.0036$). (C), the relative expression of microRNA-155 in the low O_2 saturation group was significantly higher than that in the high O_2 saturation group ($P = 0.04$). (D), the relative expression of miRNA-10b was not significantly different between the two groups ($P = 0.067$).

EMT in A549 cells that overexpress ACE2, as demonstrated in various studies [36,37]. Additionally, it has been observed that rhinovirus can interfere with adherens and tight junctions, resulting in heightened membrane permeability. This is believed to be a potential mechanism for rhinovirus-induced viremia and EMT [38,39].

The results of our study showed that the level of miRNA-21 expression in serum-derived exosomes had a significant rise. Previous research has shown that miRNA-21 as an extracellular microRNA or carried by exosomes can be considered a marker for inflammation [40,41]. Increased expression of this microRNA has also been observed in various cancers such as colon, ovarian, breast, and melanoma and inflammatory diseases such as colitis and osteoarthritis [42–45]. Besides, microRNA has been shown to augment the expression of pro-inflammatory cytokines such as IL 6, IL 1, TNF, and IL 8 [46,47]. Given that patients with COVID-19 develop cytokine secretion syndrome, it is likely that miRNA-21 will play a vital role in the pathogenesis of COVID-19. Recently, a study similar to our results reported an increase in the expression level of miRNA-21 in the serum of COVID-19 patients [48]. On the other hand, miRNA-21 plays an important role in the EMT phenomenon, which increases metastasis in cancer patients [24,49]. It is hypothesized that this miRNA may increase metastasis in patients with cancer and COVID-19. Moreover, there is a hypothesis that exosomes containing the miRNA-21 can cause pathogenic lesions in different places and even far from the exosome secretion site. Another study showed that higher expression of miRNA-21 increased TGFβ signaling, which led to fibrosis of target tissue [50]. As patients with COVID-19 develop lesions and fibrosis in their lungs, miRNA-21 is probably an essential component in developing these lesions in lung tissue (Fig. 6). Our results also showed a significant increase in the expression of miRNA-218 in the patient group compared to the

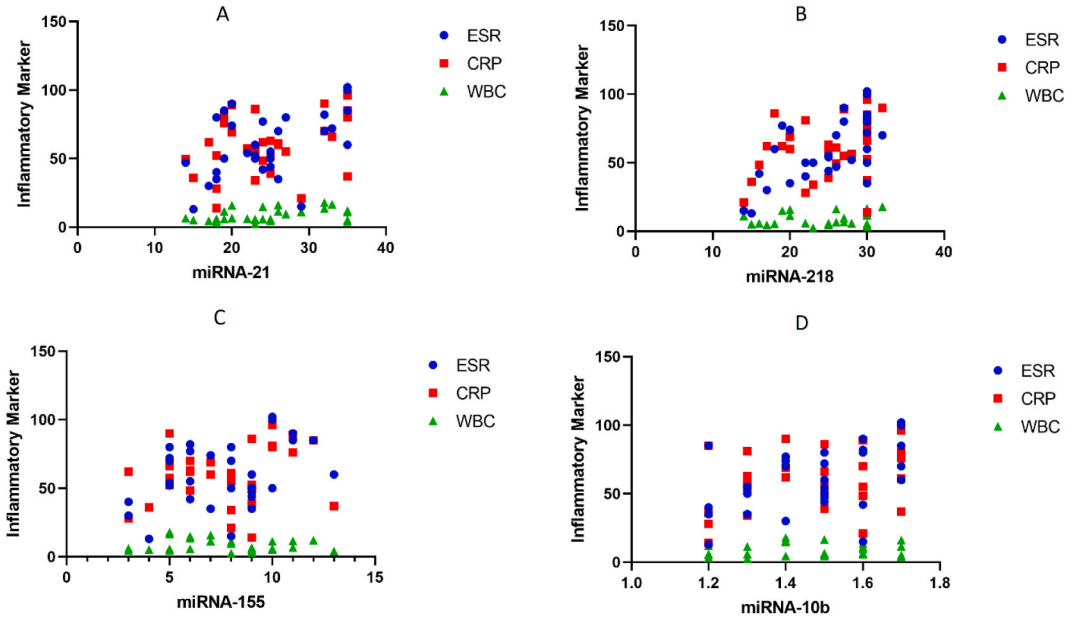

Fig. 5. Correlation between each inflammatory marker (ESR, WBC, CRP) and each microRNA.

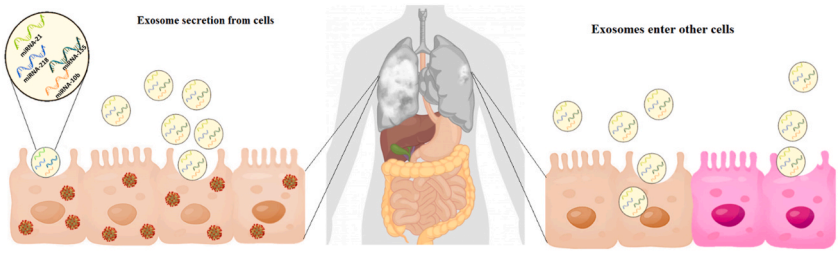

Fig. 6. A sketch of the role of exosomes containing microRNA in causing lesions elsewhere from the site of exosome formation. The secretion of exosomes containing microRNAs is involved in inflammatory processes and the EMT pathway from cells, as well as the development of lesions and pathogenic reactions elsewhere by the entry of these exosomes into other cells.

control group. miRNA-218 has been a major regulator in several types of malignancies, including gastric and colon cancers [51,52]. miRNA-218 has been shown to inhibit the production of IFN-I, a critical cytokine in the immune system against virus-infected cells, leading to the virus escaping immune system responses [53]. It is hypothesized that the SARS-COV2 virus may impair the immune response by increasing the expression of this microRNA in the cells, serum, or circulating exosomes of patients with COVID-19 On the other hand, a study has shown that inhibition of miRNA-218 reduces synovial inflammation in rats with knee osteoarthritis. In other words, miRNA-218, like miRNA-21, can play an inflammatory role [54], and possibly miRNA-218 could also induce pro-inflammatory cytokines in these patients.

We also measured the expression level of miRNA-155. Our results also showed that miRNA-155 expression was increased in serum-derived exosomes of patients with COVID-19. The role of miRNA-155 in the induction of pro-inflammatory cytokines in various diseases such as various cancers and ARDS has been proven [55–57]. There is a direct relationship between increased miRNA-155 expression and the severity of ARDS. Inhibition of miRNA-155 has been revealed to reduce pneumonia in mice infected with the respiratory virus [58]. Higher expression of this microRNA in the serum of patients with COVID-19 is consistent with our results [48]. Also, increased expression of this microRNA has been reported in SARS-COV 2 virus-infected Calu-3 lung cell lines [59]. On the other hand, studies report the role of miRNA-155 in developing EMT in various cancers such as hepatocellular carcinoma [60,61]. In this regard, due to the transport of miRNA-155 by exosomes and their transfer to other cells, it is possible that this microRNA, like miRNA-21, can cause lesions and inflammation in other cells.

The miRNA-10b was another factor that we measured in serum-derived exosomes of patients with COVID-19, and our results showed a significant difference between the control and patient groups. miRNA-10b expression is increased in various sites such as glioblastoma, pancreas and breast [62]. It has also been shown that the expression of miRNA-10b in cancer patients and cytomegalovirus infections is increased by the LMP 1 protein, a viral protein CMV. In other words, this viral infection intensifies the expression of miRNA-10b [63] and these changes in microRNA expression were also identified in our study. The influential role of miRNA-10b in the

EMT phenomenon in various cancers such as breast cancer has been proven. Higher expression of this microRNA in circulatory exosomes of patients with COVID-19 possibly activates invasive mechanisms and metastasis in patients with cancer and COVID-19 [64]. In a recent study, Mimmi and her team discovered significant differences in the expression of certain microRNAs in patients with acute phase COVID-19. They found that exosomes expressing angiotensin-converting enzyme 2 (ExoACE2) exhibited upregulation of let-7g-5p and hsa-miR-4454+miR-7975, while hsa-miR-208a-3p and has-miR-323-3p were downregulated compared to non-ExoACE2 samples. These findings shed light on potential biomarkers and therapeutic targets for COVID-19 [65]. It has been discovered that hsa-miR-4454 and miR-7975 are implicated in the invasion and migration of tumor cells. Given their elevated expression in exosomes derived from the plasma of COVID-19 patients, it is plausible to propose that these microRNAs, along with the microRNAs identified in this study, may contribute to the pathogenesis of COVID-19 and the EMT pathway [66–68].

Our empirical findings indicated that miRNA-21, miRNA-155, and miRNA-218 were significantly upregulated in individuals experiencing hypoxia (O2 saturation <90.6 %). This upregulation may imply a contributory function for these microRNAs in the pathophysiology of COVID-19, particularly via mechanisms related to the induction of inflammation and the exacerbation of hypoxic states. Furthermore, extant literature has demonstrated that hypoxic conditions can precipitate an augmented expression of these microRNAs [69–71]. Consequently, it is plausible to postulate that hypoxic conditions may enhance the expression of these microRNAs, which could subsequently facilitate inflammatory responses.

Due to the critical role of miRNA-21, miRNA-218, and miRNA-155, and miRNA-10b in the pathogenesis of COVID-19 disease, they can be used as therapeutic targets in formulating the drug. Perhaps targeting these microRNAs can prevent the progression of COVID-19 disease to acute and systemic inflammation. The high expression level of the microRNAs mentioned above in the serum-derived exosomes of people with asymptomatic COVID-19 can also be considered a biomarker.

5. Conclusion

Based on the findings of this study, the expression levels of miRNA-21, miRNA- 218, miRNA-155, and miRNA-10b are higher in serum-derived exosomes of COVID-19 patients, which can be involved in the pathogenesis of this disease. These microRNAs activate other cell signaling pathways due to their transmission by exosomes and engage other parts of the body.

CRediT authorship contribution statement

Nazanin Joudaki: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Ali Khodadadi:** Project administration, Conceptualization. **Marziye Shamshiri:** Methodology, Investigation. **Sajad Dehnavi:** Methodology, Investigation. **Ali Asadirad:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We appreciate all the people who helped us with this project. This study was supported by the Research Council of Ahvaz University of Medical Sciences.

References

[1] A.S. Fauci, H.C. Lane, R.R. Redfield, Covid-19—navigating the Uncharted, Mass Medical Soc, 2020.
[2] R. Castagnoli, M. Votto, A. Licari, I. Brambilla, R. Bruno, S. Perlini, et al., Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review, JAMA Pediatr. 174 (9) (2020) 882–889.
[3] P. Mehta, D.F. McAuley, M. Brown, E. Sanchez, R.S. Tattersall, J.J. Manson, COVID-19: consider cytokine storm syndromes and immunosuppression, The lancet 395 (10229) (2020) 1033–1034.
[4] T. Hirano, M. Murakami, COVID-19: a new virus, but a familiar receptor and cytokine release syndrome, Immunity 52 (5) (2020) 731–733.
[5] M.Z. Tay, C.M. Poh, L. Rénia, P.A. MacAry, L.F. Ng, The trinity of COVID-19: immunity, inflammation and intervention, Nat. Rev. Immunol. 20 (6) (2020) 363–374.
[6] P.E. Lazzerini, M. Boutjdir, P.L. Capecchi, COVID-19, arrhythmic risk, and inflammation: mind the gap, Circulation 142 (1) (2020) 7–9.
[7] J Gong, H Dong, Q-S Xia, Z-y Huang, D-k Wang, Y Zhao, et al., Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19: a retrospective study, BMC Infectious Diseases 20 (1) (2020) 963.
[8] R.J. Jose, A. Manuel, COVID-19 cytokine storm: the interplay between inflammation and coagulation, Lancet Respir. Med. 8 (6) (2020) e46–e47.
[9] M. Merad, J.C. Martin, Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages, Nat. Rev. Immunol. 20 (6) (2020) 355–362.
[10] I. Wortzel, S. Dror, C.M. Kenific, D. Lyden, Exosome-mediated metastasis: communication from a distance, Dev. Cell 49 (3) (2019) 347–360.
[11] D.M. Pegtel, S.J. Gould, Exosomes, Annu. Rev. Biochem. 88 (2019) 487–514.
[12] K.D. Popowski, P.U.C. Dinh, A. George, H. Lutz, K. Cheng, Exosome therapeutics for COVID-19 and respiratory viruses, View (2021) 20200186.
[13] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, cell 136 (2) (2009) 215–233.
[14] T. Sugatani, J. Vacher, K.A. Hruska, A microRNA expression signature of osteoclastogenesis, Blood, The Journal of the American Society of Hematology 117 (13) (2011) 3648–3657.

[15] Y. Mei, C. Bian, J. Li, Z. Du, H. Zhou, Z. Yang, R.C. Zhao, miR-21 modulates the ERK–MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation, J. Cell. Biochem. 114 (6) (2013) 1374–1384.
[16] A.M. Krichevsky, G. Gabriely, miR-21: a small multi-faceted RNA, J. Cell Mol. Med. 13 (1) (2009) 39–53.
[17] S. Nersisyan, N. Engibaryan, A. Gorbonos, K. Kirdey, A. Makhonin, A. Tonevitsky, The Potential Role of miR-21-3p in Coronavirus-Host Interplay, BioRxiv., 2020.
[18] A. Głobińska, M. Pawełczyk, M.L. Kowalski, MicroRNAs and the immune response to respiratory virus infections, Expet Rev. Clin. Immunol. 10 (7) (2014) 963–971.
[19] S. Jia, H. Zhai, M. Zhao, MicroRNAs regulate immune system via multiple targets, Discov. Med. 18 (100) (2014) 237–247.
[20] F.W. Lai, K.B. Stephenson, J. Mahony, B.D. Lichty, Human coronavirus OC43 nucleocapsid protein binds microRNA 9 and potentiates NF-κB activation, J. Virol. 88 (1) (2014) 54–65.
[21] B. Mallick, Z. Ghosh, J. Chakrabarti, MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells, PLoS One 4 (11) (2009) e7837.
[22] Y. Ma, C. Wang, M. Xue, F. Fu, X. Zhang, L. Li, et al., The coronavirus transmissible gastroenteritis virus evades the type I interferon response through IRE1α-mediated manipulation of the microRNA miR-30a-5p/SOCS1/3 axis, J. Virol. 92 (22) (2018).
[23] I. Georgakopoulos-Soares, D.V. Chartoumpekis, V. Kyriazopoulou, A. Zaravinos, EMT factors and metabolic pathways in cancer, Frontiers in oncology 10 (2020) 499.
[24] H. Wang, Z. Tan, H. Hu, H. Liu, T. Wu, C. Zheng, et al., microRNA-21 promotes breast cancer proliferation and metastasis by targeting LZTFL1, BMC Cancer 19 (1) (2019) 1–13.
[25] C. Ma, F. Wei, H. Xia, H. Liu, X. Dong, Y. Zhang, et al., MicroRNA-10b mediates TGF-β1-regulated glioblastoma proliferation, migration and epithelial-mesenchymal transition, Int. J. Oncol. 50 (5) (2017) 1739–1748.
[26] T.X. Lu, A. Munitz, M.E. Rothenberg, MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression, J. Immunol. 182 (8) (2009) 4994–5002.
[27] L. Yang, B. Wang, Q. Zhou, Y. Wang, X. Liu, Z. Liu, Z. Zhan, MicroRNA-21 prevents excessive inflammation and cardiac dysfunction after myocardial infarction through targeting KBTBD7, Cell Death Dis. 9 (7) (2018) 1–14.
[28] C. Liechty, J. Hu, L. Zhang, K.W. Liechty, J. Xu, Role of microRNA-21 and its underlying mechanisms in inflammatory responses in diabetic wounds, Int. J. Mol. Sci. 21 (9) (2020) 3328.
[29] G. Conickx, P. Mestdagh, F. Avila Cobos, F.M. Verhamme, T. Maes, B.M. Vanaudenaerde, et al., MicroRNA profiling reveals a role for microRNA-218-5p in the pathogenesis of chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 195 (1) (2017) 43–56.
[30] Y. Liang, Y. Feng, W. Wu, C. Chang, D. Chen, S. Chen, G. Zhen, microRNA-218-5p plays a protective role in eosinophilic airway inflammation via targeting δ-catenin, a novel catenin in asthma, Clin. Exp. Allergy 50 (1) (2020) 29–40.
[31] E. Tili, J.-J. Michaille, D. Wernicke, H. Alder, S. Costinean, S. Volinia, C.M. Croce, Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer, Proc. Natl. Acad. Sci. USA 108 (12) (2011) 4908–4913.
[32] R.M. O'Connell, D. Kahn, W.S. Gibson, J.L. Round, R.L. Scholz, A.A. Chaudhuri, et al., MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development, Immunity 33 (4) (2010) 607–619.
[33] G. Mahesh, R. Biswas, MicroRNA-155: a master regulator of inflammation, J. Interferon Cytokine Res. 39 (6) (2019) 321–330.
[34] L. Tribolet, E. Kerr, C. Cowled, A.G. Bean, C.R. Stewart, M. Dearnley, R.J. Farr, MicroRNA biomarkers for infectious diseases: from basic research to biosensing, Front. Microbiol. 11 (2020).
[35] J. Duy, J.W. Koehler, A.N. Honko, R.J. Schoepp, N. Wauquier, J.-P. Gonzalez, et al., Circulating microRNA profiles of Ebola virus infection, Sci. Rep. 6 (1) (2016) 1–13.
[36] Y.J. Lai, C.H. Chao, C.C. Liao, T.A. Lee, J.M. Hsu, W.C. Chou, et al., Epithelial-mesenchymal transition induced by SARS-CoV-2 required transcriptional upregulation of Snail, Am. J. Cancer Res. 11 (5) (2021) 2278–2290.
[37] C.A. Stewart, C.M. Gay, K. Ramkumar, K.R. Cargill, R.J. Cardnell, M.B. Nilsson, et al., Lung Cancer Models Reveal SARS-CoV-2-Induced EMT Contributes to COVID-19 Pathophysiology, bioRxiv, 2021.
[38] N.K. Yeo, Y.J. Jang, Rhinovirus infection-induced alteration of tight junction and adherens junction components in human nasal epithelial cells, Laryngoscope 120 (2) (2010) 346–352.
[39] D.M. Minor, D. Proud, Role of human rhinovirus in triggering human airway epithelial-mesenchymal transition, Respiratory research 18 (2017) 1–16.
[40] Y. Tanaka, H. Kamohara, K. Kinoshita, J. Kurashige, T. Ishimoto, M. Iwatsuki, et al., Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma, Cancer 119 (6) (2013) 1159–1167.
[41] D. V Sawant, W. Yao, Z. Wright, C. Sawyers, R. S Tepper, S. K Gupta, et al., Serum microRNA-21 as a biomarker for allergic inflammatory disease in children, MicroRNA 4 (1) (2015) 36–40.
[42] W. Yu, K. Zhu, Y. Wang, H. Yu, J. Guo, Overexpression of miR-21-5p promotes proliferation and invasion of colon adenocarcinoma cells through targeting CHL1, Molecular Medicine 24 (1) (2018) 1–12.
[43] P.M. Báez-Vega, I.M.E. Vargas, F. Valiyeva, J. Encarnación-Rosado, A. Roman, J. Flores, et al., Targeting miR-21-3p inhibits proliferation and invasion of ovarian cancer cells, Oncotarget 7 (24) (2016) 36321.
[44] B.C. Melnik, MiR-21: an environmental driver of malignant melanoma? J. Transl. Med. 13 (1) (2015) 1–16.
[45] F. Wu, M. Zikusoka, A. Trindade, T. Dassopoulos, M.L. Harris, T.M. Bayless, et al., MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2α, Gastroenterology 135 (5) (2008) 1624–1635. e24.
[46] A.J. Lakhter, R.E. Pratt, R.E. Moore, K.K. Doucette, B.F. Maier, L.A. DiMeglio, E.K. Sims, Beta cell extracellular vesicle miR-21-5p cargo is increased in response to inflammatory cytokines and serves as a biomarker of type 1 diabetes, Diabetologia 61 (5) (2018) 1124–1134.
[47] M. Borja-Gonzalez, J.C. Casas-Martinez, B. McDonagh, K. Goljanek-Whysall, Inflamma-miR-21 negatively regulates myogenesis during ageing, Antioxidants 9 (4) (2020) 345.
[48] Garg A, Seeliger B, Derda AA, Xiao K, Gietz A, Scherf K, et al. Circulating cardiovascular microRNAs in critically ill COVID-19 patients Short title: microRNA signatures in COVID-19. Eur. J. Heart Fail.
[49] N. Petrovic, miR-21 might be involved in breast cancer promotion and invasion rather than in initial events of breast cancer development, Mol. Diagn. Ther. 20 (2) (2016) 97.
[50] R.T. Marquez, S. Bandyopadhyay, E.B. Wendlandt, K. Keck, B.A. Hoffer, M.S. Icardi, et al., Correlation between microRNA expression levels and clinical parameters associated with chronic hepatitis C viral infection in humans, Lab. Invest. 90 (12) (2010) 1727–1736.
[51] Y. Ye, B. Gu, Y. Wang, S. Shen, W. Huang, E2F1-mediated MNX1-AS1-miR-218-5p-SEC61A1 feedback loop contributes to the progression of colon adenocarcinoma, J. Cell. Biochem. 120 (4) (2019) 6145–6153.
[52] J. Tie, Y. Pan, L. Zhao, K. Wu, J. Liu, S. Sun, et al., MiR-218 inhibits invasion and metastasis of gastric cancer by targeting the Robo1 receptor, PLoS Genet. 6 (3) (2010) e1000879.
[53] Y. Qiu, X. Geng, J. Ban, Y. Liu, MicroRNA-218 inhibits type I interferon production and facilitates virus immune evasion via targeting RIG-I, Biotechnol. Appl. Biochem. 67 (3) (2020) 396–403.
[54] H. Liao, Z. Zhang, Z. Liu, W. Lin, J. Huang, Y. Huang, Inhibited microRNA-218-5p attenuates synovial inflammation and cartilage injury in rats with knee osteoarthritis by promoting sclerostin, Life Sci. 267 (2021) 118893.
[55] E. Vigorito, S. Kohlhaas, D. Lu, R. Leyland, miR-155: an ancient regulator of the immune system, Immunol. Rev. 253 (1) (2013) 146–157.
[56] Z. Wu, R. Hao, P. Li, X. Zhang, N. Liu, S. Qiu, et al., MicroRNA expression profile of mouse lung infected with 2009 pandemic H1N1 influenza virus, PLoS One 8 (9) (2013) e74190.

[57] P.S. Woods, L.M. Doolittle, L.E. Rosas, S.P. Nana-Sinkam, E. Tili, I.C. Davis, Increased expression of microRNA-155-5p by alveolar type II cells contributes to development of lethal ARDS in H1N1 influenza A virus-infected mice, Virology 545 (2020) 40–52.
[58] E. De Smet, H. Van Eeckhoutte, F.A. Cobos, E. Blomme, F. Verhamme, S. Provoost, et al., The role of miR-155 in cigarette smoke-induced pulmonary inflammation and COPD, Mucosal Immunol. 13 (3) (2020) 423–436.
[59] E. Wyler, K. Mösbauer, V. Franke, A. Diag, L.T. Gottula, R. Arsie, et al., Bulk and Single-Cell Gene Expression Profiling of SARS-CoV-2 Infected Human Cell Lines Identifies Molecular Targets for Therapeutic Intervention, BioRxiv, 2020.
[60] X. Kong, F. Liu, J. Gao, MiR-155 promotes epithelial-mesenchymal transition in hepatocellular carcinoma cells through the activation of PI3K/SGK3/β-catenin signaling pathways, Oncotarget 7 (40) (2016) 66051.
[61] F. Liu, X. Kong, L. Lv, J. Gao, TGF-β1 acts through miR-155 to down-regulate TP53INP1 in promoting epithelial–mesenchymal transition and cancer stem cell phenotypes, Cancer letters 359 (2) (2015) 288–298.
[62] A. Lund, miR-10 in development and cancer, Cell Death Differ. 17 (2) (2010) 209–214.
[63] G. Li, Z. Wu, Y. Peng, X. Liu, J. Lu, L. Wang, et al., MicroRNA-10b induced by Epstein-Barr virus-encoded latent membrane protein-1 promotes the metastasis of human nasopharyngeal carcinoma cells, Cancer letters 299 (1) (2010) 29–36.
[64] X. Han, S. Yan, Z. Weijie, W. Feng, W. Liuxing, L. Mengquan, F. Qingxia, Critical role of miR-10b in transforming growth factor-β1-induced epithelial–mesenchymal transition in breast cancer, Cancer Gene Ther. 21 (2) (2014) 60–67.
[65] S. Mimmi, A.M. Zimbo, S. Rotundo, E. Cione, N. Nisticò, A. Aloisio, et al., SARS CoV-2 spike protein-guided exosome isolation facilitates detection of potential miRNA biomarkers in COVID-19 infections, Clin. Chem. Lab. Med. 61 (8) (2023) 1518–1524.
[66] H. Wang, H. Hu, Z. Luo, S. Liu, W. Wu, M. Zhu, et al., miR-4454 up-regulated by HPV16 E6/E7 promotes invasion and migration by targeting ABHD2/NUDT21 in cervical cancer, Biosci. Rep. 40 (9) (2020) BSR20200796.
[67] Y. Zheng, A. Song, Y. Zhou, Y. Zhong, W. Zhang, C. Wang, et al., Identification of extracellular vesicles-transported miRNAs in Erlotinib-resistant head and neck squamous cell carcinoma, Journal of cell communication and signaling 14 (2020) 389–402.
[68] E.G. Park, D.H. Lee, W.R. Kim, Y.J. Lee, W.H. Bae, J-m Kim, et al., Human endogenous retrovirus-H-derived miR-4454 inhibits the expression of DNAJB4 and SASH1 in non-muscle-invasive bladder cancer, Genes 14 (7) (2023) 1410.
[69] C.Z. Angel, M.Y.C. Stafford, C.J. McNally, H. Nesbitt, D.J. McKenna, MiR-21 is induced by hypoxia and down-regulates RHOB in prostate cancer, Cancers 15 (4) (2023).
[70] U. Bruning, L. Cerone, Z. Neufeld, S.F. Fitzpatrick, A. Cheong, C.C. Scholz, et al., MicroRNA-155 promotes resolution of hypoxia-inducible factor 1alpha activity during prolonged hypoxia, Mol. Cell Biol. 31 (19) (2011) 4087–4096.
[71] J. Liu, Y. Li, L. Lyu, L. Xiao, A.A. Memon, X. Yu, et al., Integrin α5 is regulated by miR-218-5p in endothelial progenitor cells, J. Am. Soc. Nephrol. 33 (3) (2022) 565–582.