

Plasma microRNAs levels associate with the outcome of ARDS patients

Qin Sun^a, Yingzi Huang^{b*}

^{a, b} Department of Critical Care Medicine, Zhongda Hospital, School of Medicine, Southeast University, No.87, Dingjiaqiao Road, Gulou District, Nanjing 210009, China

Abstract

Background:

Mesenchymal stem cells (MSC) are stromal cells with renew ability for multilineage differentiation. Therefore MSC has been considered as a therapy method for rebuilding alveoli structure and repairing the function of pulmonary vascular endothelial cells (VEC). However, whether MSC can play a therapeutic role in ARDS patients depends on different ARDS phenotypes. Recent studies about microRNAs (miRNAs) proved that miRNAs played important roles in MSC regulating function and activity of VEC which may affect therapeutic response and outcome to different ARDS patients, but few studies focused on this field. The purpose of our study is that plasma miRNAs regulated VEC's function and specific miRNAs which were related to MSC and VEC (MSC-VEC-miRNAs) expressed differently between survival and non-survival ARDS patients. We aim to find specific MSC-VEC-miRNAs which are associated with the outcome of ARDS patients.

Methods: We obtained *MSC-VEC*-miRNAs through searching PUBMED database. A number of 101 ARDS patients were screened and 57 of them were included in our research within 24 hours of admission to ICU. We then collected their clinical data and their blood samples, then we did real-time PCR to test plasma levels of *MSC-VEC*-miRNAs.

Results: Fourteen *MSC-VEC*-miRNAs were selected in this study. We included 57 ARDS patients and 18(31.6%) of them died on Day 28 after diagnosis. Plasma miR-26a level in non-survival group was significantly lower than that in survival group (0.33[0.09-1.17] vs. 0.97[0.17-3.49], $P=0.046$). Plasma miR-320 level in non-survival group was significantly higher than that in survival group (0.37[0.16-1.66] vs. 0.18[0.07-0.39], $P=0.041$). There was no statistical difference of other 12 miRNA between two groups.

Conclusion: Plasma miR-26a and miR-320 levels have a certain predictive value for the prognosis of ARDS patients.

1. Background

Acute respiratory distress syndrome (ARDS) is a fatal syndrome which is characterized by acute lung injury due to uncontrolled inflammatory response, mismatch of ventilation to perfusion, and inflexible hypoxemia. Mesenchymal stem cells (MSC), a kind of multipotent stromal cells, can preserve vascular endothelial integrity and attenuate pulmonary vascular permeability which has been proved in many studies in ARDS [1-3]. MicroRNAs (miRNAs) are a class of small noncoding RNAs which can regulate gene expression levels and inhibiting their translation [4-6]. Previous studies [7,8] showed that MSC could regulate miRNAs levels, which then influence downstream target genes and affect pulmonary VEC's function. Based on above researches, we speculated that specific miRNAs were closely associated with therapeutic effects of MSC and outcome in ARDS patients.

In this article, we temporarily gave the *MSC-VEC*-

miRNAs a definition as a bunch of miRNAs which can regulate VEC functions through a miRNA-MSC-VEC axis in ARDS. After consulting relevant literatures about MSC in ARDS, we found 14 *MSC-VEC*-miRNAs from previous researches [9,10]. We then design specific primers and examined these 14 *MSC-VEC*-miRNAs expression in plasma which were obtained from ARDS patients. We then compared the difference of *MSC-VEC*-miRNAs levels between ARDS patients with different prognosis. Different expressed *MSC-VEC*-miRNAs may provide potential diagnosis and treatment targets in the future.

2. Methods

2.1 Patients

We screened all patients came to ICU in our hospital which is a tertiary hospital in Jiangsu Province from Jan

^a sunqin1990seu@126.com

^b *Correspondence: yz_huang@126.com

first 2016 to Sep 31, 2016. Adult patients admitted to ICU who met ARDS Berlin criteria were included in our study. The exclusion criteria was as follows: meet ARDS criteria more than 24 hours, immunosuppressive medication using, malignant tumor patients and pregnant women. All patients included in our research received conventional therapy in our center. Mechanical ventilation, fluid management and vasoactive drug were decided by clinicians.

Considering that we get patients' blood samples in our research in a department of critical care medicine, which may lead to ethics issues. We made our protocol approved by an Institutional Ethics Committee with an approval Number 2016ZDSYLL034.0. We also registered this trail on clinicaltrials.gov (Registration No. NCT02885675).

2.2 Data collection

Demographic information such as age, sex, BMI, underline diseases, causes of ARDS, and clinical and laboratory characteristics like pH, PaO₂, PaCO₂, P/F were collected. APACHE II, SOFA score and murray scores were calculated or obtained directly from our electronic medical record system using data within 24 h after admission. The patients were followed-up till 28 days and the outcome at 28 days was recorded as 28-day mortality.

2.3 Sample acquisition and RNA isolation

ARDS patients' blood samples were collected within 24 hours after diagnosis. We then centrifuged all blood samples at a speed of 1900×g for 10 minutes. After centrifugation, the blood was divided into two layers, the supernatant was absorbed and frozen in the refrigerator at -80 degrees Celsius. Then we reheated all frozen plasma

at 37°C in a water bath and isolated RNA from a RNA extraction kits and reverse-transcribed them into DNA. We designed primers from Primer Bank and a fixed qPCR system was used to get CT values. GAPDH was used as internal parameter. $\Delta \Delta CT$ was calculated to get fold changes of all miRNAs.

2.4 Statistical analysis

In this study we use SPSS 20 (Chicago, IL, USA) to finish all statistical analyses. If the variables were continuous and normally distributed, they were presented as means \pm standard deviation. Student's t-tests were used to compare the difference of variables between two groups. Mann-Whitney U tests was used in non-normally distributed continuous variables. *P*-values < 0.05 were considered statistically significant in this study.

3. Results

3.1 Baseline characteristics of included patients

During our research phase, 101 ARDS patients were screened in our ICU. After excluding 44 patients, 57 ARDS patients were included in final analysis, among whom 18 died before Day 28. We presented patients' baseline characteristics in Table 1. From this table, no significant difference was shown between survival and non-survival group on Age, BMI, underlying diseases and the causes of ARDS. Disease severity which was demonstrated by APACHE II score, SOFA score, incidence of septic shock were significant higher in non-survival group relative to survival group.

Table 1 Baseline characteristics of ARDS patients after inclusion

Variable	Total (n=57)	Survival group(1) (n=39)	Non-survival group(2) (n=18)	P Value
Basic characteristics				
Age (Years)	49.0 \pm 17.5	46.3 \pm 17.3	54.4 \pm 17.1	0.10
Male n(%)	41(71.9%)	32(82.1%)	9(50%)	0.02
BMI	23.9 \pm 3.6	24.0 \pm 3.5	23.6 \pm 3.9	0.64
APACHE II	21.3 \pm 8.4	18.7 \pm 7.1	26.9 \pm 8.5	<0.001
SOFA	10.4 \pm 4.9	8.9 \pm 4.5	13.7 \pm 3.9	<0.001
Underlying diseases				
COPD n(%)	1(1.8%)	1(2.6%)	0(0%)	1.00
Hypertension n(%)	16(28.1%)	12(30.8%)	4(22.2%)	0.50
Coronary heart disease n(%)	8(14.0%)	3(7.7%)	5(27.8%)	0.11
Cerebrovascular disease n(%)	8(14.0%)	5(12.8%)	3(16.7%)	1.00
Diabetes n(%)	12(21.1%)	6(15.4%)	6(33.3%)	0.23
Hepatic or gall diseases n(%)	7(12.3%)	5(12.8%)	2(11.1%)	1.00
Casues of ARDS				
Pneumonia n(%)	36(63.2%)	22(56.4%)	14(77.8%)	0.12
Inhalation of gas/liquid n(%)	3(5.3%)	3(7.1%)	0(0%)	0.56
Pulmonary contusion n(%)	4(7%)	4(10.3%)	0(0%)	0.40

Sepsis n(%)	3(5.3%)	2(5.1%)	1(5.6%)	1.00
Pancreatitis n(%)	4(7.0%)	3(7.7%)	1(5.6%)	1.00
Others n(%)	7(12.3%)	5(12.8%)	2(11.1%)	1.00
Severity of lung injury				
PH	7.4[7.35-7.45]	7.4[7.36-7.46]	7.4[7.32-7.44]	0.61
FiO2	0.5[0.4-0.6]	0.5[0.4-0.6]	0.5[0.4-0.78]	0.53
PEEP(cmH2O)	8[5-12]	8[5-10]	9[5-12.5]	0.22
P/F(mmHg)	165[112-211]	168[117-225]	136[97-198]	0.14
Murray Scores	2.3[1.7-3.1]	2.3[1.7-2.7]	2.8[2.3-3.7]	0.01
Lac	2.1[1-3.1]	1.7[0.9-2.4]	3.1[1.2-5.2]	0.01

3.2 Differential plasma levels of miRNAs in two groups

Plasma miR-26a level in non-survival group was 0.18[0.07-0.39] fold change, which was significantly

lower than survival group (0.18[0.07-0.39] vs. 0.37[0.16-1.66], $P=0.046$). However, plasma miR-320 level in non-survival group was significantly higher than that in survival group (0.97[0.17-3.49] vs. 0.33[0.09-1.17], $P=0.041$). There was no statistical difference of other 12 miRNA between two groups (Figure 1).

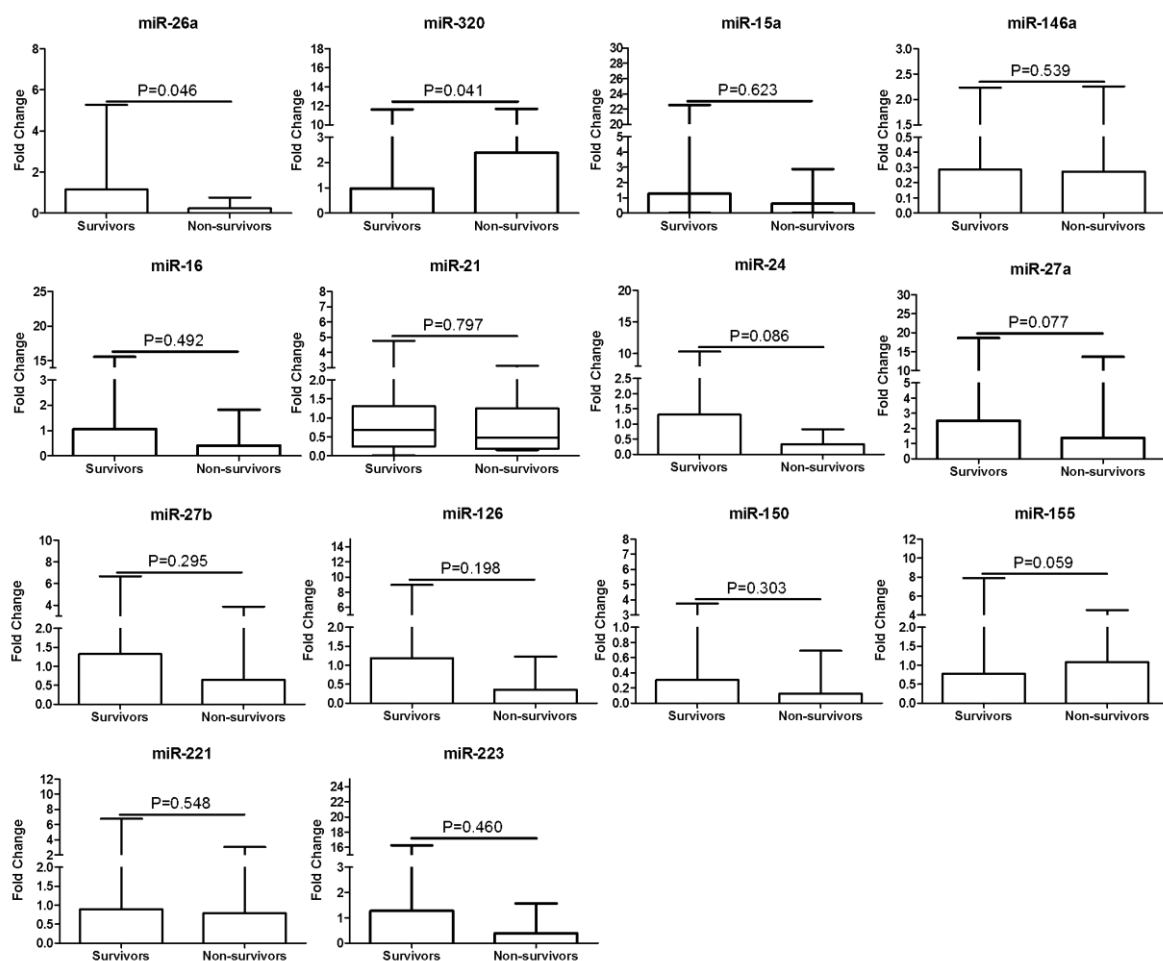


Fig 1. Expression levels of *MSC-VEC* miRNAs in survival and non-survival group.

4. Limitations

Some limitations were not negligible in this study. The subjects of this study were a group of ARDS patients with multiple etiologies of lung injury, which lead to significant heterogeneity in the etiology of ARDS. The primary disease itself may be the reason for miRNA changes, which may affect plasma miRNA levels.

5. Conclusion

Plasma miR-26a and miR-320 levels have a certain predictive value for the prognosis of ARDS patients.

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