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The exosome of platelet endothelial cell adhesion molecule-1 (PECAM1) protein

A potential risking star in high blood pressure patients (HBPP)

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Abstract

A number of studies have demonstrated that exosomes were involved in important physiological and pathological processes through cell-to-cell communication in cardiovascular disease, which contained nucleic acids, proteins, and lipid contents. In our study, we found that the protein platelet endothelial cell adhesion molecule-1 (PECAM1) was an extracellular vesicle in the blood of high blood pressure patients (HBPP).

Isolated the vesicles from the blood of HBPP and health examiners and detected its size and morphology with nanoparticle tracking analysis, then we identified its surface protein CD63, CD81, and the protein expression of PECAM1 in the exosome with western blot. Furthermore, we analyzed the correlation between the expression of PECAM1 and the high blood degree with linear regression analysis.

Our results showed that the morphology of extracellular vesicles was more evident in high blood pressure groups than healthy controls, and the protein expression of PECAM1 was also abundant in the vesicles of HBPP, however, there were no extracellular vesicles in the blood samples of healthy controls. Besides, linear regression showed the linear correlation coefficient $R=0.901$, $P<.01$ between the expression of PECAM1 and the systolic blood pressure of the high blood patients. Therefore, the exosome of protein of PECAM1 was a potential risking star in HBPP.

Abbreviations: eNOS = nitric oxide (NO) synthase, HBPP = high blood pressure patients, PECAM1 = platelet endothelial cell adhesion molecule-1.

Keywords: extracellular vesicles, high blood pressure, platelet endothelial cell adhesion molecule-1

1. Introduction

The exosomes were lipid bilayer membranous vesicles with a diameter 30 to 150 nm and a density of 1.13 to 1.21 g/mL, which contained miRNA, lncRNA, protein, lipids, and DNA materials.[1] They played an important role in physiological and pathological processes through cell to cell communication. In recent years, with the next-generation sequencing technology has been widely used in bioscience research, the exosomes have gradually been identified in cardiovascular disease, besides, they were thought to be with great potential in clinical for the significance of risk prediction, as well as biomarkers for high blood pressure and atherosclerosis management.[2] For example, Chunhua Shi[3] identified that the heat shock protein 27 in extracellular vesicles was a potential anti-inflammatory therapy in cardiovascular disease. Besides, both Mengmeng Lu[4] and Xiaoyan Liu[5] proved that circulating miRNAs in exosomes was a functional diagnostic biomarkers, which had the potential for predicting the outcomes in vascular inflammation, high blood pressure, and atherosclerosis. While in our study, we found that the protein platelet endothelial cell adhesion molecule-1 (PECAM1) in the extracellular vesicles was abundant in the blood samples of high blood pressure patients (HBPP). The PECAM1 was expressed on platelets and leukocytes and it was primarily concentrated at the borders of endothelial cells in blood vessel, which had been shown to be involved in endothelial cells mechanosensing, namely being a signal transducer of flow-mediated Gab1 tyrosine phosphorylation and being an activator of its downstream signals of Akt and endothelial nitric oxide (NO) synthase.[6,7] Hence, during the process of endothelial dysfunction, Gab1 transduced the signals of cytokine and growth factor receptors,[8] and the activation of eNOSwas impaired, which induced high blood pressure,[9,10] therefore, in this study, we attempted to study the roles of protein PECAM1 in the extracellular vesicles of HBPP.

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The peripheral blood was collected from the patients and volunteers of our hospital and the consent was orally and was written before collecting the blood. All participants were agreed to sign the informed consent and the study was approved by our Hospital Ethics Committee.

The authors have no funding and conflicts of interest to disclose.

The analytical data were available on request from the corresponding author.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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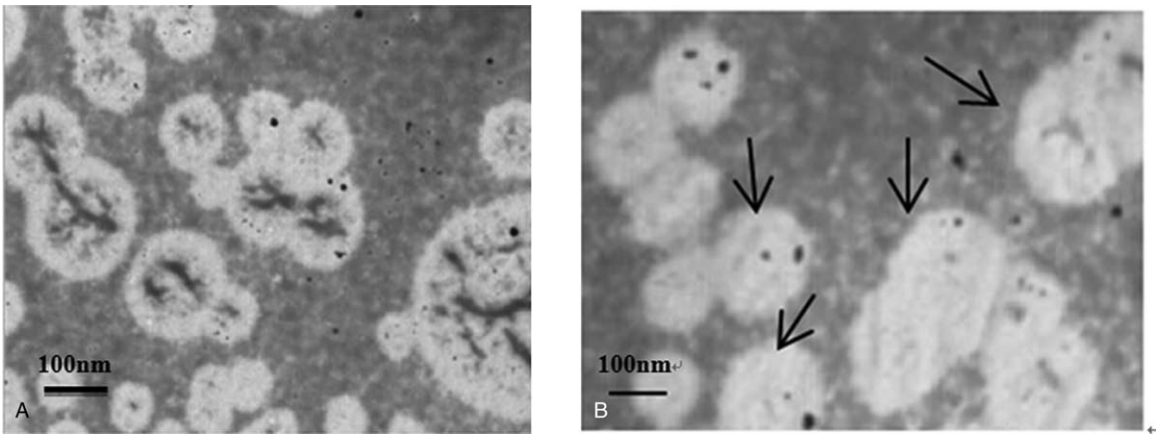

Figure 1. The protein PECAM1 in the exosomes harvested from the blood of high blood pressure patients. (A) The derived exosomes imaged with NTA at a diameter in the range of 50 to 100 nm. Scale bar, 100 nm. (B) Arrows indicate PECAM1 immunogold labeling (black dots) on the exosome surface. Scale bars, 100 nm. PECAM1 = platelet endothelial cell adhesion molecule-1.

2. Materials and methods

2.1. Ethics statement

The peripheral blood was collected from the patients and volunteers of our hospital and the consent was orally and was written before collecting the blood. All participants were agreed to sign the informed consent and the study was approved by our Hospital Ethics Committee.

2.2. Inclusion criteria

Age: 40 to 55 years old,

Systolic blood pressure: 140 mm Hg to 159 mm Hg and (or) diastolic blood pressure:90 mm Hg to 99 mm Hg, besides, the patients were not received any treatment at diagnose,

The patients with no other metabolic disease and infectious disease, except for high blood pressure disease.

2.3. Exclusion criteria

The patients with serious disease and metabolic disease,

The age is not suitable,

The systolic blood pressure is over 159 mm Hg and diastolic blood pressure is over 99 mm Hg,

The patients quit in the study.

2.4. Reagents and antibodies

Anti-CD63 (abcam, ab193349), anti-CD81 (abcam, ab109201), anti-PECAM1 (abcam, ab28364), anti-β-actin (abcam, ab8227), RIPA buffer (Solebo, Beijing), protease inhibitor cocktail PMSF (Beyotime), PVDF membrane (Bio-Rad, Hercules, CA), BCA assay kit (Thermo, PICPI23223).

2.5. Blood collection and exosome isolation

Twenty blood samples of patients with high blood pressure and fifteen blood samples of healthy controls were collected from July 15th, 2018 to October 30th, 2018. Besides, the included patients were all conformed to the inclusion criteria and exclusion criteria. Then, the blood samples were centrifuged for 15 minutes at 3000 × g, 4°C within 30 minutes. The upper plasma was carefully transferred to a new tube and centrifuged for 15 minutes at 3000 × g, 4°C to remove additional cellular fragments and debris. Then, the cleared supernatant was carefully transferred to a new tube and ultracentrifuged at 30,000 rpm for 30 minutes at 4°C. The obtained pellet was resuspended in sterilized phosphate-buffered saline.

2.6. Exosome particle size analysis

The full size and its morphology of the exosomes extracted from the blood samples was measured by Nanoparticle tracking analysis (NTA) (NanoSight NTA 2.3, Malvern Instruments Ltd, UK)

2.7. Western blot analysis

The total protein was extracted from exosomes using lysis buffer containing protease inhibitor cocktail PMSF and the protein concentration was examined with the BCA assay kit. Afterward, the proteins were separated using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF membrane (Bio-Rad, Hercules, CA). Then the membrane was incubated in 5% nonfat milk for 1 hour and incubated overnight at 4°C with primary antibody homo anti-CD63 (1:2000, Abcam), homo anti-CD81 (1:2000, Abcam), homo anti-PECAM1 (1:2000, Abcam), homo anti-β-actin (1:2000, Abcam). Then incubated with rabbit polyclonal

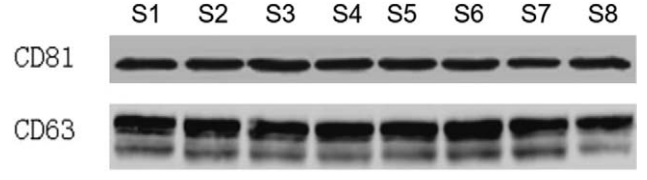

Figure 2. The surface marker protein was extracted from exosomes and analyzed with western blot, the results revealed that the exosomal markers CD81 and CD63 were obiviously expressed on the exosome surface (samples: S1–S8).

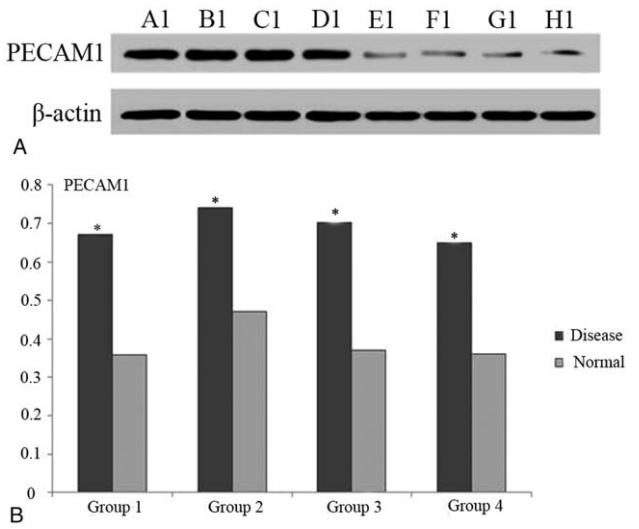

Figure 3. The expression of PECAM1 in the exosome of the blood samples were analyzed by Western Blot (A: the representative samples in the high blood patients vs the healthy control) (high blood patients' samples: Group 1–4: A1-B1-C1-D1; healthy control's samples: Group 1–4: E1-F1-G1-H1). And the difference was analyzed with GraphPad Prism 6.0 software, (B) in the 4 representative groups, the expression of PECAM1 protein in the exosome was higher than the healthy controls, *, &, # represented with significance ($P < .05$). PECAM1 = platelet endothelial cell adhesion molecule-1.

antibody at 4°C for 60 minutes and washed the membranes 3 times for 10 minutes.

Subsequently, the bands were determined using Image J 1.46 r (NIH, Bethesda, MD) for scan analysis.

2.8. The linear regression assay

The linear regression analyzed the correlation between the expression PECAM1 in the exosome and the systolic blood pressure of HBPP.

2.9. Statistics

The significant analysis was performed using SPSS17.0 software, and GraphPad Prism 6.0 software was used to perform the figure legends. The statistical significance between groups was compared with ANOVA method, $P < .05$ standard for significance.

3. Results

3.1. Exosome particle size analysis and protein analysis

The exosome pellets had a diameter in the range of 50 to 100 nm estimated by the NTA (Fig. 1). The exosome protein content from the supernatant fraction and the exosomal pellets were analyzed by Western blotting using the exosomal markers (CD81, CD63) and the exosome protein PECAM1, the results revealed that the exosomal markers CD81 and CD63 were obviously expressed on the exosome surface (Fig. 2) and the expression of PECAM1 in the exosome was obviously higher than the healthy control, and there was little PECAM1 expression in the healthy control (Fig. 3).

3.2. The linear regression assay

The expression of PECAM1 in the exosome of the blood samples was analyzed by Western Blot, and the blood pressure of the

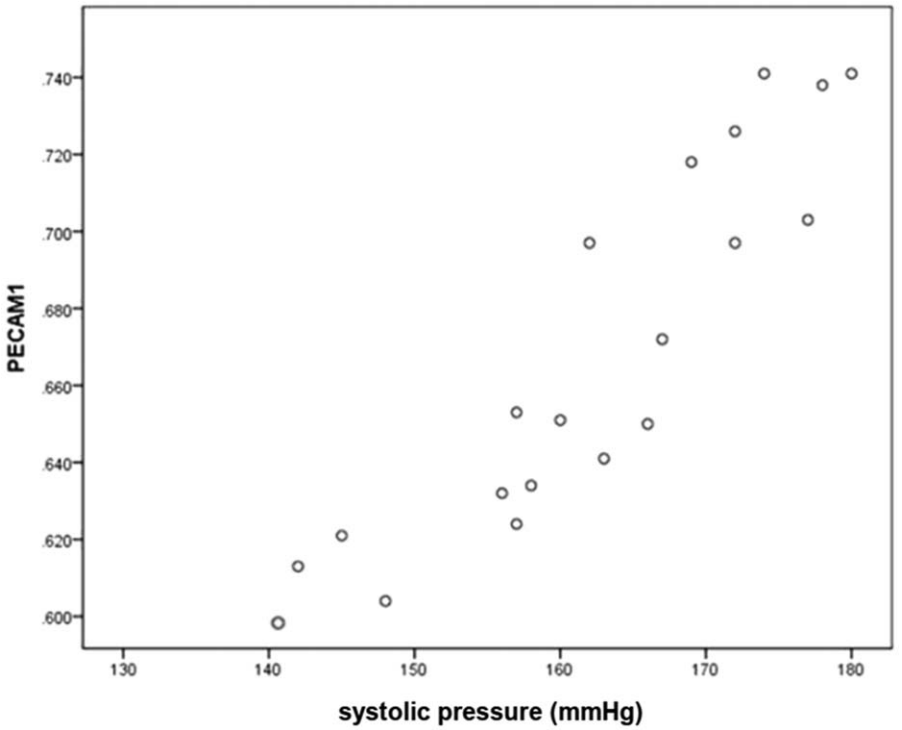

Figure 4. The correlation between the expression PECAM1 in the exosome and the systolic pressure of the high blood pressure patients, the expression PECAM1 was higher in high blood pressure patients and there was significant correlation in each patient' blood sample, the linear coefficients $R = 0.901$, $P < .01$. PECAM1 = platelet endothelial cell adhesion molecule-1.

included patients was recorded. The correlation between the expression PECAM1 in the exosome and the systolic pressure of the HBPP demonstrated that the expression PECAM1 in the exosome was abundant in HBPP and there was significant correlation in each patient blood sample, its linear coefficients $R = 0.901$, $P < .01$. (Fig. 4).

4. Discussion

Our studies revealed that extracellular vesicles of the protein PECAM1 were evident in HBPP . And there was significant correlation between the expression PECAM1 in the exosome and the systolic pressure of the HBPP . Studies showed that PECAM1 could induce monocyte adhesion in the endothelial or vascular wall cells to develop plaque and reduce IL-10 plasma concentration, thus accelerating vascular endothelial injury.[11,12] Moreover, the protein VE-Cadherin and PECAM-1 were found involving in the activity of tension induced vascular remodeling.[13] Therefore, the exosome PECAM1 could play important roles in the pathological processes of high blood pressure, which has the potential to be a risking star in HBPP. PECAM-1 expressed on platelets and leukocytes was identified to regulate transendothelial migration of monocytes[14] and neutrophils.[15] Harry et al[16] found that PECAM-1 in the mechanosensory complex containing PECAM-1-VE-cadherin-VEGFR2-NF-κB was critically important for atherosclerotic lesion development in the aortic arch of $Apoe^{-/-}$ mice. It was suggested that the dynamic fluid shear stress generates tension between adjacent endothelial cells and activates PECAM-1, which led to nuclear translocation of NF-κB and expression of inflammatory and adhesive mediators, such as VCAM-1 to promote blood pressure and atherosclerosis.[13] While Ren et al[17] indicated that PECAM1 played an important role in the maintenance of the HUVEC monolayer barrier integrity through the IL-13-pSTAT6-PECAM1 complex. Besides, Xu et al[18] showed that the activated VEGFR2 could recruit Gab1 and the phosphorylation of Gab1 leading to recruitment of PI3K, which then transmit a flow signal to Akt and eNOS and subsequently induced NO production in ECs to the involvement of the pathology of cardiovascular disease. Additionally, in the study of Dautova[19] declared that Calcium phosphate particles were critically important for exosome release in human vascular smooth muscle cells through releasing IL-1β. Hence, based on these findings, much more further studies are needed to reveal which triggered the release of the exosome PECAM1 and what is the pathology mechanism of how extracellular vesicles PECAM1 induced hypertension. Then, the exosome PECAM1 will be more meaningful in the physiological and pathological processes of high blood pressure disease.

Author contributions

Haiming Shi wrote the manuscript and Yun Shi conducted most of the experiments, Yun Shi, Haiming Shi collected the data and analyzed the data and all authors approved the submission.

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