MicroRNA-98 can serve as a diagnostic marker for congenital heart disease-associated pulmonary artery hypertension

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Congenital heart disease (CHD) has become the leading mortal cause for an infant from congenital abnormalities. Pulmonary artery hypertension (PAH) is one of the complications of CHD.[1] It has also been reported that upregulation of microRNA-98 (miR-98) could mediate the suppression of cardiac hypertrophy, which implies that miR-98 might play an important role in CHD.[2] In this study, the clinical data from hospitalized patients were retrospectively analyzed to identify if there is any evidence of diagnosis of circulating miR-98 on CHD patients with PAH.

This was a single-center, retrospective study performed in Beijing Anzhen Hospital. Data of patients admitted to the hospital from December 2016 to December 2017 were collected. Sixty cases of CHD were diagnosed by color echocardiography. And patients with the following comorbidities were excluded: pulmonary disease, heart valve disease, heart failure, hematological diseases, human immunodeficiency virus infection, and severe liver and renal insufficiency. The study got the approval of the Clinical Ethical Committee of the Beijing Anzhen Hospital (No. KY2017-051-03).

Color Doppler echocardiography was used to estimate pulmonary artery systolic pressure (PASP) according to the tricuspid regurgitation pressure difference.[3]

The cardiac index (CI), heart rate and stroke volume of each patient were detected using a VBP-10 Doppler cardiovascular detection kit.

The morning fasting radial artery blood was collected from all patients, and the total RNA was extracted by TRIzol one-step method (Invitrogen, Carlsbad, CA, USA) after serum separation. Reverse transcription real-time polymerase chain reaction (RT-qPCR) was performed as per the instructions of a qPCR kit (Thermo Fisher Scientific, Shanghai, China). The relative expression of miR-98 was normalized by U6. The quantitative primer of miR-98 was: 5'-GCTGAGGTAGTAAGTTGTATTG-3' (forward primer) and 5'-CAGTGCGTGTCGTGGAGT-3' (reverse primer), and the quantitative primer of U6 was 5'-GTGCGTGTCGTGGAGTCG-3' (forward primer) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse primer).

Statistical analyses were performed using SPSS 19.0 software (IBM Corp. Armonk, NY, USA). Measurement data were expressed as means ± standard deviation. One-way analysis of variance (ANOVA) method was used for the multi-group comparison (the homogeneity test of variance was conducted before analysis), followed by Tukey's multiple comparison test. The pairwise comparison of the multiple groups/mean values was performed using the unpaired-*t* test, and the correlation analysis was conducted using Pearson correlation test. The receiver-operating characteristic (ROC) curve was drawn to calculate the sensitivity, specificity and area under the curve (AUC). A two-tailed $P < 0.05$ was considered statistically significant.

As shown in Supplementary Table 1, http://links.lww.com/CM9/A372, the gender, age, CI, heart rate, and stroke volume of the 60 patients (28 males and 32 females, 2 months–10 years) showed no significant difference (all $P > 0.05$), indicating a comparability among the participants.

According to the PASP measured by echocardiography, 60 children with CHD were assigned into two groups: CHD-non-PAH group (pulmonary artery mean pressure (PAMP) <25 mmHg ($n = 15$, eight males and seven females, with the mean age of 4.96 ± 1.93 years); CHD-PAH group (PAMP ≥ 25 mmHg, $n = 45$, 20 males and 25 females, with

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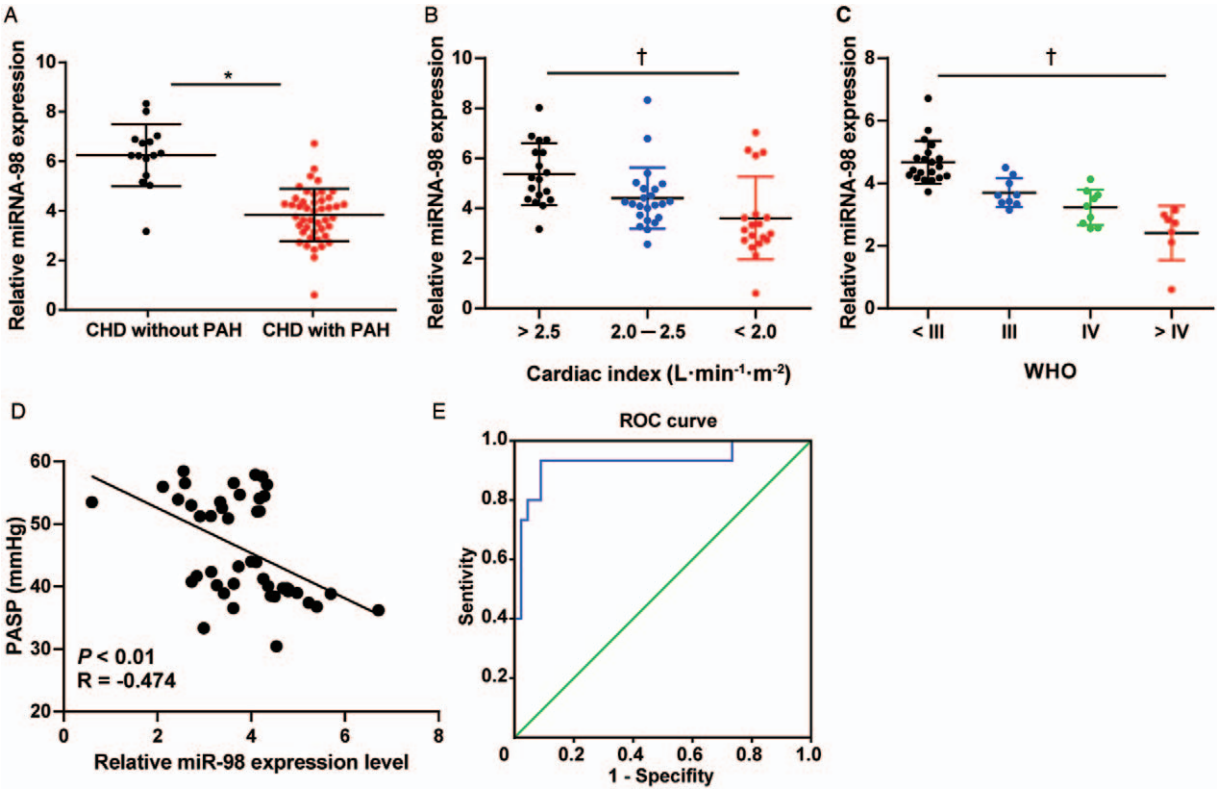

Figure 1: miR-98 is lowly expressed in CHD with PAH patients and negatively correlated with PAH severity. (A) miR-98 expression in all CHD patients detected using RT-qPCR; (B) analysis of the correlation between CI and PASP in all CHD patients; (C) miR-98 expression in CHD patients with different PAH stages (according to the WHO standard) detected using RT-qPCR; (D) correlation analysis between miR-98 and PASP in all CHD-PAH patients; (E) ROC curve analysis of the concentration of miRNA-98 for PAH diagnosis, with the AUC = 0.817 (95% CI, 0.667–0.967), $P < 0.001$. In panel A, unpaired *t* test was applied for data analysis, while in panels B and C, one-way ANOVA and Tukey's multiple comparison test was applied, and in panel D, Pearson's correlation test was used. $^{*}P < 0.05$. AUC: area under the curve; CHD: Congenital heart disease; CI: Cardiac index; PAH: Pulmonary artery hypertension; PASP: Pulmonary artery systolic pressure; ROC: receiver operating characteristic; RT-qPCR:Reverse transcription real-time polymerase chain reaction.

the mean age of 5.54 ± 2.06 years). There was no significant difference in age and gender between the two groups. Furthermore, the patients in the CHD-PAH group were allocated into < III, III, IV and >IV grade according to the PAH grading standards of WHO.

Serum miR-98 expression in CHD-PAH patients was markedly lower than that in CHD without PAH patients ($P < 0.05$). Higher expression of miR-98 was related to higher CI. Lower expression of miR-98 was correlated with higher severity of PAH according to the WHO grading in 45 CHD-PAH patients, and miR-98 expression was negatively correlated with PASP of PAH patients. The ROC curve and AUC suggested the 95% confidence interval of miR-98 for CHD-PAH was 0.929 (95% [confidence interval] CI, 0.833–1.000, $P < 0.001$), while the sensitivity and specificity was 91.1% and 93.3%, respectively; the positive predictive value and negative predictive value were 97.62% and 77.78%, respectively (all $P < 0.05$) [Figure 1A–E].

Reduced miR-98 levels and pulmonary artery endothelial cells proliferation are common features observed in PAH.[4] Our study identified that higher expression of miR-98 was related to higher CI, and miR-98 expression was lower in CHD-PAH patients than that in CHD-non-PAH patients. miR-98 is also regarded as a negative-feedback mediator against other forms of cardiac hypertrophy, and the upregulation of miR-98 inhibits cardiac hypertrophy to control heart failure and pathological hypertrophy.[2] Therefore, we may conclude that miR-98 is correlated with the PAH in CHD.

Upregulated PASP is correlated with elevated mortality in individuals with and without prevalent cardiovascular diseases. We further investigated the relationship between serum miR-98 expression and PASP through correlation analysis, and found that miR-98 expression was negatively correlated with PASP in CHD complicated with PAH.

PAH is a devastating disease with multiple pathophysiological hallmarks including excessive pulmonary vasoconstriction, inflammation, right ventricular hypertrophy (RVH) and right ventricular systolic pressure (RVSP).[5] miR-98 expression is decreased in hypoxia-exposed pulmonary artery endothelial cells, and miR-98 plays a key role in PAH pathogenesis, suggesting miR-98 as a promising strategy to improve PAH therapeutics.[4] Our results elicited that lower expression of miR-98 was correlated with higher severity of PAH according to the WHO grading in 45 CHD-PAH patients. The above findings provide evidence for the correlation between miR-98 and PASP and PAH.

Moreover, the later results of ROC curve analysis in this study suggested that the 95% confidence interval of

miR-98 for CHD-PAH was 0.929 (95% CI, 0.833–1.000, $P < 0.001$), while the sensitivity and specificity indicate that miR-98 was negatively correlated with PASP, and upregulation of miR-98 might be a positive regulator in the treatment of PAH in CHD patients.

To sum up, our study provided evidence that miR-98 was poorly expressed in CHD-PAH patients, and it could serve as a diagnostic marker for CHD-PAH. We hope these findings could provide novel insights into PAH, especially in CHD-associated PAH prevention and treatment. Hopefully, more studies in this field would be conducted in the near future to validate our findings, and to figure out the potential mechanism implicated.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s)/patient's guardians has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the article. The patients/patient's guardians understand that their names and initials will not be published and due efforts will be made to conceal the identity of the patient, although anonymity cannot be guaranteed.

Conflicts of interest

None.

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