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Diagnostic Value of Let-7a-5p in Essential Hypertension

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Keywords: essential hypertension | let-7a-5p | renin-angiotensin-aldosterone system

ABSTRACT

This study aimed to investigate the role of let-7a-5p in the pathogenesis of essential hypertension (EH) and its correlation with the renin-angiotensin-aldosterone system (RAAS) biomarkers. Ninety-eight EH patients and 24 healthy controls (HC) enrolled in the study were assayed for the relative expression of let-7a-5p in plasma by quantitative real-time polymerase chain reaction (Q-PCR), and biomarkers of the RAAS system, including angiotensin-converting enzyme 2 (ACE2), Ang (1-7), MAS1, angiotensin-converting enzyme (ACE), angiotensin II (Ang II), and angiotensin II type 1 receptor (AT1R), were determined by enzyme-linked immunosorbent assay (ELISA) The expression levels of the biomarkers of RAAS system were determined. The results showed that the expression levels of let-7a-5p in the plasma of EH patients were remarkably higher than those of HC. The prediction model of combined let-7a-5p showed high accuracy by constructing a subject operating characteristic (ROC) curve with an area under the curve (AUC) of 0.885, and the reliability of the model was further verified by the Hosmer–Lemeshow (H–L) goodness-of-fit test, the Model Calibration Curve, and the Decision Curve Analysis. Spearman correlation analysis revealed that the expression of let-7a-5p was positively correlated with ACE ($r = 0.352$, $p < 0.001$), and mediation analysis indicated that ACE partially mediated between let-7a-5p and the development of hypertension. The present study concludes with the potential of let-7a-5p as a companion diagnostic biomarker for EH. It suggests that there may be a complex regulatory mechanism between it and specific RAAS biomarkers, which provides a new perspective on the pathogenesis and diagnosis of EH.

1 | Introduction

Elevated arterial pressure in the physical circulation is a characteristic of essential hypertension (EH), one of the most prevalent chronic cardiovascular disorders. A major threat to global health, hypertension not only raises the risk of stroke and cardiovascular and cerebrovascular disorders, but it also damages target organs including the heart, brain, and kidneys and, in extreme situations, can result in multi-organ failure [1, 2]. About 90% more people currently suffer from hypertension than there were a few years ago, and the morbidity and mortality rates of cardiovascular illnesses linked to hypertension are rising annually as well [3]. Tragically, blood pressure control rates are still declining despite this dire circumstance. Additionally, the pathophysiology of EH is yet unclear. Its pathogenesis is intricate and complex, involving overactivation of the renin-angiotensin-aldosterone system [4, 5] (RAAS), abnormal hyperactivation of the sympathetic nervous system [6, 7], and vascular remodeling [8, 9], in which the RAAS system plays an integral role. Angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2) are key enzymes in the RAAS system. Angiotensin I (Ang I) is transformed into angiotensin II (Ang II) by ACE to fulfill what it does

in vascular remodeling and the development of atherosclerosis, among other things. A basic metabolic method, the ACE/Ang II /AT1R axis arises when Ang II interacts with the angiotensin II type 1 receptor (AT1R). But in the last few years, scientists have discovered distinct perspectives ACE2/Ang (1-7)/Mas axis that is completely different from the classical axis [10, 11]. The peptide angiotensin-7Ang (1-7), which is created when ACE2 breaks down Ang II, reverses the vasoconstrictor effects of Ang II. In the RAAS system, this new finding adds significantly to the complexity of the RAAS system and provides new perspectives for a deeper understanding of the pathogenesis of hypertension.

MicroRNAs are a class of small-molecule noncoding RNAs about 21–23 nucleotides long with the ability to interact with multiple target genes [12, 13]. Numerous fundamental biological processes, including important facets of cell growth and proliferation, have been demonstrated to depend on them. Due to the stability of miRNAs in plasma and serum, they can be important in the field of disease diagnosis, which predicts that miRNAs may become a new biomarker. In the meanwhile, several miRNAs, including microRNA-136 [14] and microRNA-155 [15], are extensively involved in controlling the RAAS system and, by affecting the expression and functionality of essential system components, contribute significantly to the pathophysiology of EH. Furthermore, let-7a-5p can target and regulate Wnt/β-catenin signaling, which affects the development of idiopathic pulmonary hypertension [16]. Notably, there has not been a thorough investigation of the possible relationship between let-7a-5p and EH, which suggests new lines of inquiry. In this study, To generate new concepts for the diagnosis and treatment of EH, we examined the expression level of let-7a-5p in the plasma of EH patients, investigated its affiliation with biochemical markers of RAAS, and employed bioinformatics techniques to speculate on the roles of let-7a-5p in the occurrence, progression, or biological functions of the disease.

2 | Materials and Methods

2.1 | Samples and Controls

Our study cohort consisted of 98 hospitalized patients with EH and 24 healthy controls (HC) who were admitted to the First Affiliated Hospital and the Second Affiliated Hospital of Anhui University of Traditional Chinese Medicine from March 2022 to October 2022. The following were the requirements for inclusion: (1) age over 18 years old, regardless of gender; The diagnostic criteria of *the 2020 ISH International Hypertension Practice Guideline* were met. Systolic blood pressure (SBP) ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg while sitting for three consecutive different days; (2) confirmation by two or more attending physicians. The following were the exclusion criteria: (1) Based on their medical history, physical examination, and laboratory results, patients with cancerous hypertension and subsequent hypertension were excluded; (2) Within 6 months, significant valvular heart disease, cardiomyopathy, and unstable angina; (3) patients with severe kidney disease and liver dysfunction; (4) pregnant and lactating women; (5) patients with malignant tumors or accompanied by obvious other systemic diseases; (6) not easy to follow-up due to disability or other reasons; and (7) patients with a large number of comorbidities (>5). Depending on the presence or absence of comorbidities the hypertensive group could continue to be categorized into 65 patients in the hypertensive without comorbidities group and 33 patients in the hypertensive with comorbidities group. Twenty-four healthy individuals who had normal examination results and no other diseases during the same period were selected as the HC group.

2.2 | Clinical Data Collection

The basic clinical data of all patients were obtained from the hospital medical record system through the patient's admission number.

2.3 | Sample Collection

Samples of fasting blood were taken the first morning following admission. After routine disinfection, 4 mL blood samples were collected from the median cubital vein using an anticoagulant tube. After blood collection, the samples were centrifuged at 3000 bpm and 4°C for 10 min, and the supernatant was removed by pipetting gun into 2 mL frozen tubes and frozen in −80°C refrigerator for further examination.

2.4 | RNA Extraction and Quantitative Real-Time-Polymerase Chain Reaction (q-PCR) Detection

Trizol reagent (Thermo Fisher Scientific, US) was used to extract total RNA, that had been further reverse-transcribed into complementary DNA (cDNA). qPCR was performed three times using the fluorescence quantitative PCR kit (Invitrogen products) by the manufacturer's instructions. The relative gene expression was assessed using 2-ΔΔCt, and U6 served as an internal reference for miRNA expression analysis. The let-7a-5p primers were designed as follows: 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACTAT-3′(reverse) and 5′-GGCGGTGAGGTAGTAGGTTGT-3′(forward)

2.5 | ELISA Test for RAAS Biochemical Markers

Enzyme-linked immunosorbent assay (ELISA)-Quantizing kits (R&D Systems, Minneapolis, MN, USA) were used to measure the plasma levels of ACE2, Ang(1–7), MAS1, ACE, Ang II, and AT1R by the manufacturer's instructions.

2.6 | Statistical Analysis

Plotting and statistical analysis were performed using SPSS 26.0, GraphPad Prism 10, and R4.4.2 statistical software. The measurement data were compared using the χ^2 test, and a predictive model for hypertension was constructed using binary logistic regression analysis. The Welch's *t*-test was used when the measures were normally distributed with varying sample sizes, and the Mann–Whitney *U* test was used when they did not conform to normality. The area under the people's work characteristics (ROC) curve (AUC) was used to assess the model's discriminate

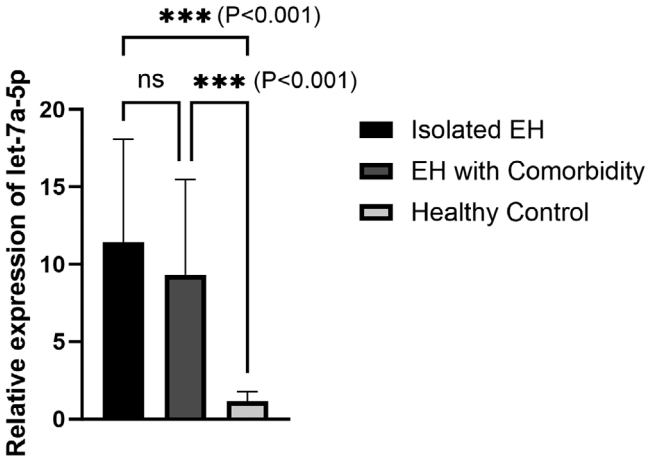

FIGURE 1 | Expression levels of let-7a-5p in isolated EH, EH with comorbidity, and HC groups. EH, essential hypertension; HC, healthy control.

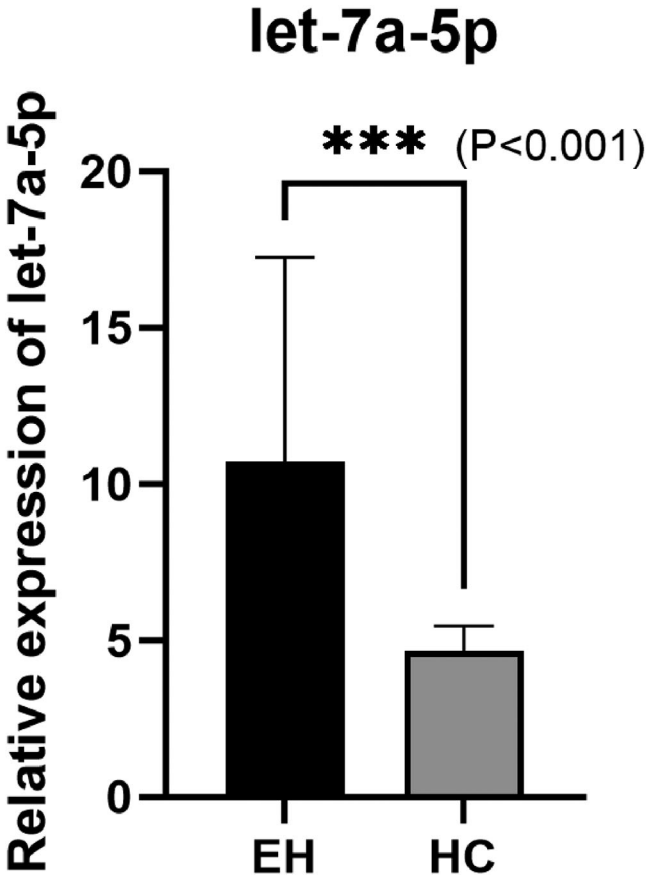

FIGURE 2 | Expression levels of let-7a-5p in EH patients and HC groups. EH, essential hypertension; HC, healthy control.

performance. The Hosmer–Lemeshow (H–L) goodness-of-fit test and Bootstrapping resampling were applied to verify the model fit and calibration correspondingly. Decision curve analysis (DCA) was used to assess the utility of the model in medical practice. Correlation analysis was performed using the Spearman or Pearson Correlation Test based on whether the data conformed to normal dissemination. The impact of mediation was analyzed using the bootstrap confidence interval (CI) approach, and the variances were deemed statistically noteworthy at $p < 0.05$ (two-sided).

TABLE 1 | Association of the expression level of let-7a-5p with EH.

Variables	OR (95% CI)	*p* value
Age	1.043 (0.992–1.096)	0.099
BMI	0.988 (0.935–1.044)	0.663
Gender	0.306 (0.080–1.164)	0.082
Smoking	0.232 (0.065–0.832)	0.025*
Drinking	0.259 (0.068–0.982)	0.047*
Family history of hypertension	2.198 (0.708–6.827)	0.173
let-7a-5p	1.338 (1.127–1.590)	0.001**

Abbreviation: EH, essential hypertension.
*denotes $p < 0.05$, ** denotes $p < 0.002$, *** denotes $p < 0.001$.

TABLE 2 | Clinical characteristics of EH patients and HC.

Variables	EH ($n = 98$)	HC ($n = 24$)	*p* value
Age (years)	62.79 ± 12.72	57.71 ± 10.05	0.071[a]
BMI (kg/m^2)	24.410 (22.22, 27.36)	23.15 (21.83, 24.47)	0.035*[,c]
Gender			0.145[b]
Male	53 (54.10%)	9 (37.50%)	
Female	45 (45.90%)	15 (62.50%)	
Smoking			0.021*[,b]
No	76 (77.60%)	13 (54.20%)	
Yes	22 (22.40%)	11 (45.80%)	
Drinking			0.059[b]
No	79 (80.60%)	15 (62.50%)	
Yes	19 (19.40%)	9 (37.50%)	
Family history of hypertension			0.044*[,b]
No	47 (48.0%)	17 (70.80%)	
Yes	51 (52.0%)	7 (29.20%)	

Note: Data are expressed as mean ± SD, median (25th, 75th quartiles), or percentages.
Abbreviations: BMI, body mass index; EH, essential hypertension; HC, healthy controls.
[a]Mann–Whitney *U* test to determine how EH patients and controls differ from one another.
[b]Chi-square test for the difference in the distribution frequencies between EH patients and controls.
[c]Student's *t*-test for the difference between EH patients and controls.
*denotes $p < 0.05$, ** denotes $p < 0.002$, *** denotes $p < 0.001$.

3 | Results

3.1 | Comparison of the Plasma Let-7a-5p Levels Among Groups

There was no significance between plasma let-7a-5p in patients with comorbidities in EH and patients without EH ($p = 0.200$), whereas both were different in the HC group, respectively

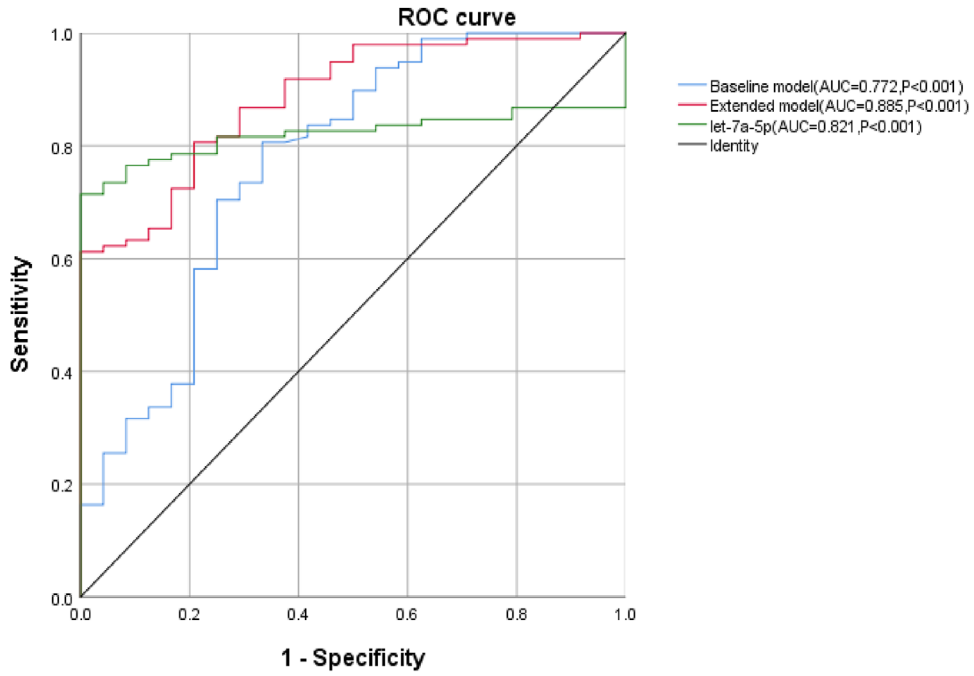

FIGURE 3 | ROC curves to evaluate the diagnostic value of baseline, extended models, and let-7a-5p for EH. EH, essential hypertension; ROC, receiving operating characteristic.

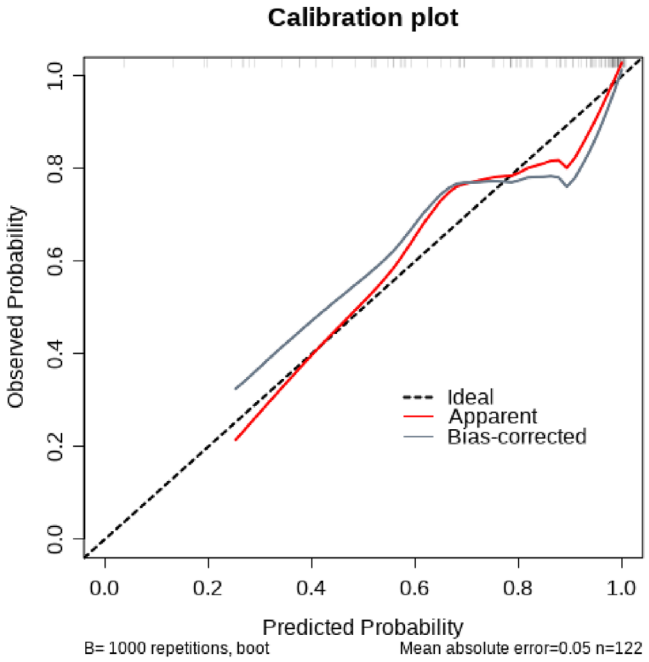

FIGURE 4 | Model calibration curve.

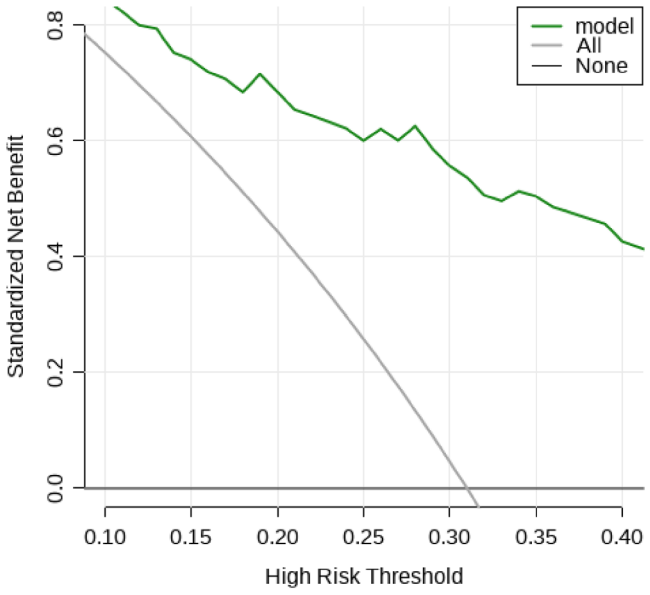

FIGURE 5 | Modeling clinical decision curves.

($p < 0.001$, Figure 1). Thus, the two groups of hypertension with no significant difference were combined into the EH group in the later statistical analysis. Compared to the HC group, the EH patients had substantially greater plasma let-7a-5p relative expression($p < 0.001$, Figure 2). Based on logistic regression analysis, let-7a-5p levels were linked to a higher chance of getting EH (adjusted OR: 1.338; 95% CI: 1.127–1.590; $p = 0.001$, Table 1) after correcting for age, gender, smoking, drinking, family history of hypertension, and BMI.

3.2 | Patient Clinical Characteristics

A total of 24 healthy individuals and 98 patients with EH were included in this study. Body mass index (BMI), smoking history, and family history were compared between the two groups, and the differences were statistically significant ($p < 0.05$). However, there were no appreciable variations between EH patients and HC in terms of age, gender, or history of alcohol use ($p > 0.05$, Table 2).

3.3 | Performance Analysis of Combined Let-7a-5p Hypertension Prediction Model

Age, sex, drinking history, smoking history, family history, and BMI were combined into one base model (baseline model) based on multifactorial logistic regression analysis. The area under the ROC curve was greater than 0.5 for both the baseline model and the addition of the let-7a-5p predictive model (extended model) as well as for let-7a-5p alone, and the diagnostic value of the predictive model with the combination of let-7a-5p was higher (AUC = 0.885, Figure 3). The H-L goodness-of-fit test showed that there was no significant difference between the predicted probability and the actual probability of occurrence (X^2 = 3.454, p = 0.903), indicating a good fit. Validation with 1000 internal samples using the Bootstrap method showed that the error between the model-predicted probability and the actual value was 0.05, indicating that the predicted probability of hypertension was in good agreement with the actual probability (Figure 4). Decision curve analysis further showed that the net benefit of the predictive model was higher than the other two extreme curves, indicating the utility and value of the model in clinical practice (Figure 5).

3.4 | Correlation Between Let-7a-5p Expression and RAAS Biochemical Markers

To determine whether let-7a-5p and RAAS biochemical markers are correlated, correlation analysis was done. According to the results of the normality test, we chose different correlation analysis methods: ACE, Ang II, and AGTR1 were analyzed by Spearman's analysis because they did not meet normality; ACE2, Ang (1-7), and MAS1 were analyzed by Pearson's analysis because they met normality. ACE (r = 0.352, p < 0.001) (Figure 6) was positively correlated by Spearman correlation analysis. While ACE2 (r = −0.085, p = 0.355), Ang (1-7) (r = 0.075, p = 0.410), MAS1 (r = 0.029, p = 0.750), Ang II (r = 0. 163, p = 0.070), and AGTR1 (r = 0.004, p = 0.960) had no correlation.

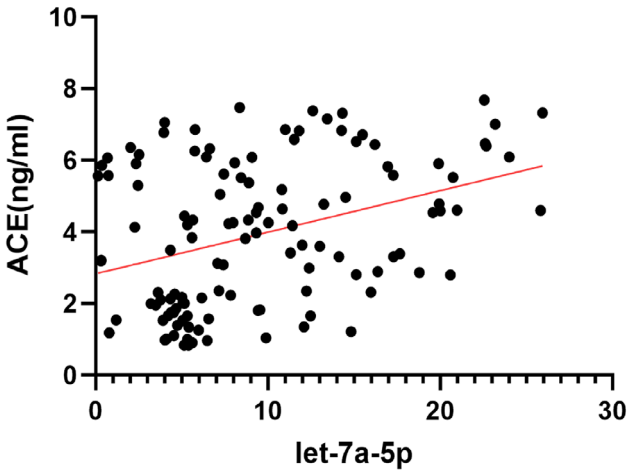

FIGURE 6 | Correlation of plasma let-7a-5p level with ACE in patients with EH. ACE, angiotensin-converting enzyme; EH, essential hypertension.

3.5 | The Mediating Effect of RAAS Biochemical Markers on the Relationship Between Plasma Let-7a-5p Levels and Hypertension

When we analyzed ACE, which correlates with let-7a-5p, for mediation, let-7a-5p partially mediated with ACE (95% BootCI: 0.098–0.258) (Table 3).

3.6 | Bioinformatics Analysis of Let-7a-5p Target Gene and Its Regulatory Pathway

Using the online databases miRPathDB 2.0, miRWalk, and miRTarBase, the number of target genes projected to be regulated

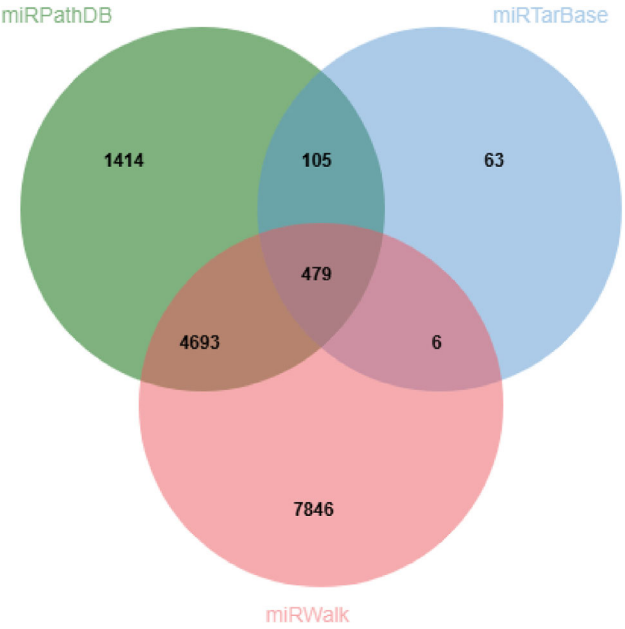

FIGURE 7 | Gene prediction of let-7a-5p target genes using miRPathDB 2.0, miRWalk, and miRTarBase.

TABLE 3 | Mediation of the relationship between plasma let-7a-5p levels and hypertension by ACE and ACE2.

Variables	*a*	*b*	*a*b* (95% BootCI)	*c'* Direct effect	Conclusion
X = >ACE =>*Y*	0.116**	0.095**	0.098 ~ 0.258	0.013*	Partial mediation

Notes: X: let-7a-5p. Y: Whether you have high blood pressure.*a* denotes the regression coefficient when X is on ACE, *b* denotes the regression coefficient when ACE is on Y, and *a*b* is the product of *a* and *b*, that is, the mediating effect, *c'* denotes the regression coefficient when *X* is on *Y* (when there is a mediating variable in the model), that is, direct effect.

Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2.

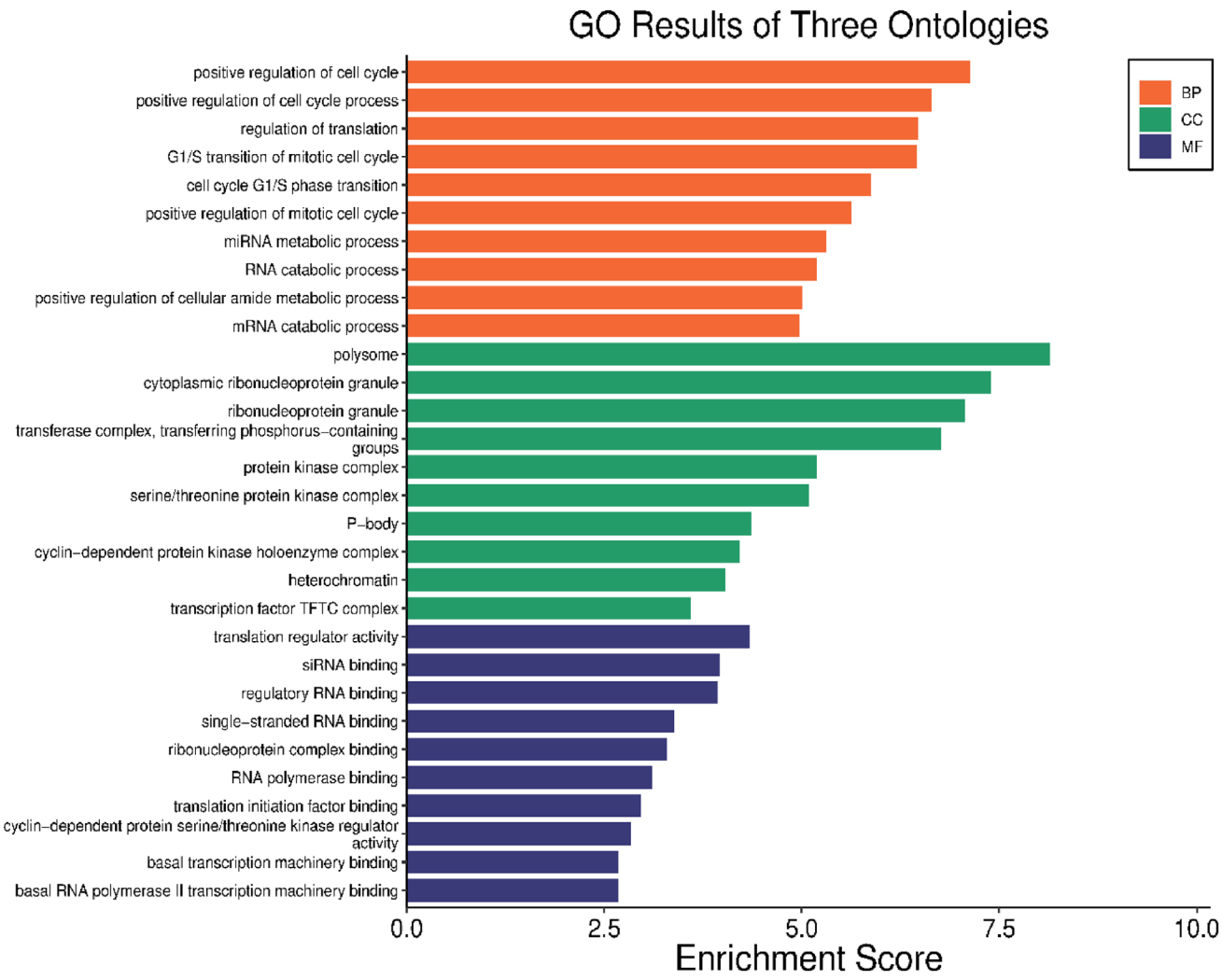

FIGURE 8 | TOP 10 GO entries each in number of genes for BP, CC, and MF. BP, biological processes; CC, cellular components; MF, molecular functions.

by let-7a-5p was 6691, 13024, and 653, respectively. The three databases also identified 479 common target genes for let-7a-5p. (Figure 7). Four hundred and seventy-nine common predicted target genes were further analyzed by GO and KEGG through the online website "Wei Sheng Xin" (https://www.bioinformatics.com.cn). GO enrichment analysis revealed that the functions were primarily concentrated in BP, which includes the G1/S transition of the mitotic cell cycle, positive regulation of the cell cycle, regulation of transition, and positive regulation of the cell cycle process; CC includes polysome, cytoplasmic ribonucleoprotein granule, and ribonucleoprotein granule, among other things; MF includes siRNA binding, regulatory RNA binding, translation regulation activity, and so on (Figure 8). KEGG results showed that let-7a-5p target genes were mainly involved in microRNAs in cancer, P53 signaling pathway, FoxO signaling pathway, and so on (Figure 9).

4 | Discussion

EH is a complex multifactorial disease resulting from environmental and genetic interactions, and its treatment is often long and complex. Some studies have shown that it is possible to achieve a reduction in blood pressure, or even a reversal of the hypertensive state, in patients with a first diagnosis of hypertension through lifestyle modifications [17]. Therefore, early diagnosis is essential for the treatment of EH. miRNA is involved in almost all biological functions, and this broad regulatory role makes it a key player in many biological processes. In recent years, there has been increasing evidence that miRNAs may be involved in the pathogenesis of EH. microRNA-21 is closely associated with hypertension [18], and miR-181a increases blood pressure by regulating renin levels in vivo [7]. Using simple assays and bioinformatics research, we investigated the role of let-7a-5p in EH and potential regulatory pathways.

First, we subdivided the diseased group into hypertension with comorbidities and hypertension without comorbidities. Upon in-depth analysis, we found that these two groups did not exhibit significant differences in plasma let-7a-5p levels. Nevertheless, it is worth noting that both the hypertension with comorbidities group and the hypertension without comorbidities group showed significant differences in plasma let-7a-5p levels compared with the HC group. Given that there was no significant difference

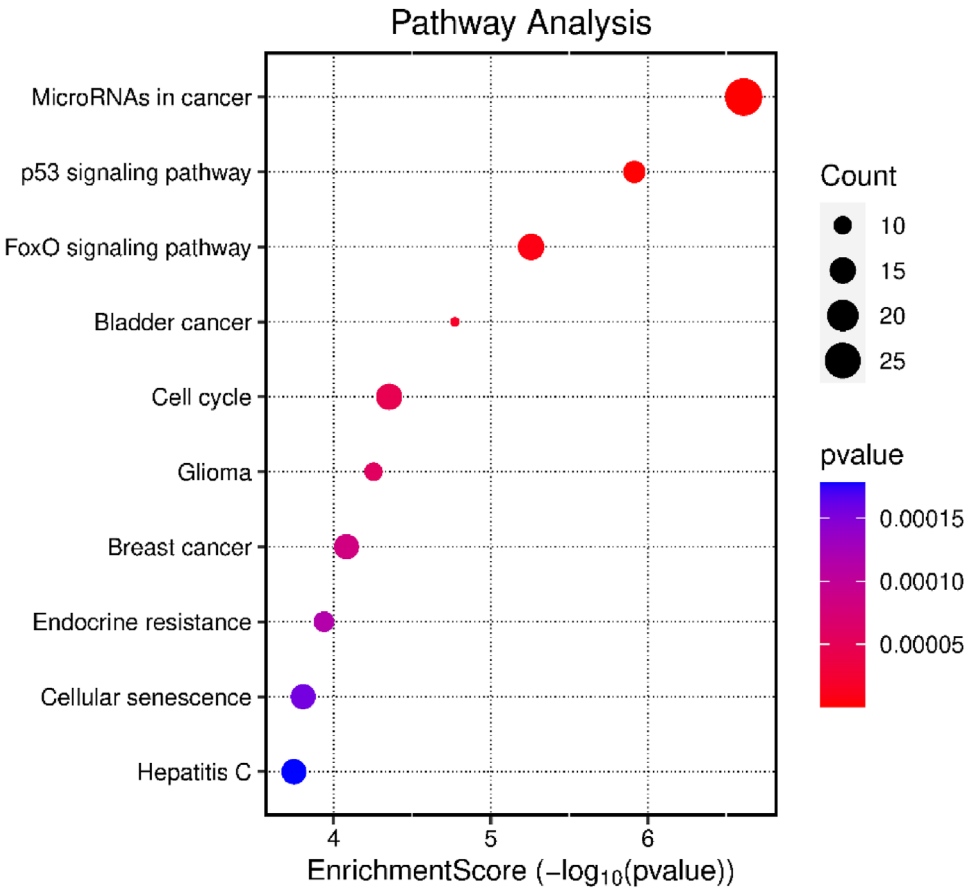

FIGURE 9 | Top 10 enriched KEGG pathways.

in plasma let-7a-5p levels between these two groups, we subsequently combined these two prevalent groups into one larger group for a more in-depth follow-up analysis. In contrast, our case–control study of the samples revealed that the relative expression of plasma let-7a-5p was significantly different between the EH and healthy groups and was upregulated in the EH. Several studies have shown that the combination of seven miRNAs, including let-7a-5p, can well differentiate between ischemic and non-ischemic heart failure etiologies [19], while let-7a-5p plays an important role in Lamin A/C (LMNA)-associated dilated neoplasia [20]. About this, we have concluded that let-7a-5p plays an indispensable part in the pathophysiology of EH. Second, we performed a multifactorial logistic regression analysis, which showed that after correcting for traditional influences such as age, sex, and drinking history, the risk of EH was elevated by 33.8% for each unit increase in the relative expression of let-7a-5p, which helped to assess the independent predictive value of let-7a-5p for the risk of EH. Meanwhile, the superiority of the hypertension prediction model constructed through logistic regression was demonstrated by validating the model using ROC curves, the Hosmer–Lemeshow test, calibration curves, and clinically applicable curves. These results conclusively suggest that let-7a-5p may be a new biomarker to aid in the diagnosis of EH.

RAAS, a classical circulating enzyme pathway, profoundly influences blood pressure regulation through a variety of mechanisms including promoting vasoconstriction, increasing sodium retention, and inducing endothelial dysfunction [21]. Although biomarkers such as ACE and ACE2 in the RAAS system function primarily at the tissue level, plasma ACE and ACE2 levels remain clinically important. Plasma ACE and ACE2 activity is a consequence of the shedding effect of protein hydrolysis in cell membranes, thus providing an opportunity to non-invasively track activation of the pathway. Li et al. [22] showed that blood microRNA 202–3p prevented Ang II from producing sST2, protecting EH; miR-483-3p [23] regulated the balance and homeostasis of ACE, ACE2, and AT2R levels, suggesting that miRNAs may have a powerful function in regulating the RAAS system. Meanwhile, let-7a-5p can directly target the 3' untranslated region (3'UTR) of ACE2, thus inhibiting ACE2 expression [24, 25]. We hypothesized that let-7a-5p might use the RAAS system to control blood pressure. Six biochemical indicators in the RAAS system were associated with let-7a-5p to validate the hypothesis. We determined that let-7a-5p had a significant relationship with ACE. A mediation effect analysis of ACE, which correlates with plasma let-7a-5p levels, was then performed, yielding that let-7a-5p partially mediates the effect with ACE. This reinforces that let-7a-5p may be involved in the pathogenesis of EH through the RAAS system. Finally, we predicted the let-7a-5p target genes using the online prediction tools miRPathDB 2.0, miRWalk, and miRTarBase and then carried out GO and KEGG enrichment analyses to gain a better understanding of the function mechanism of let-7a-5p in cells. The target genes of let-7a-5p were found to be engaged in transcriptional regulatory activities, positive regulation of the cell cycle, and other processes by GO analysis. This suggests that let-7a-5p may be implicated in the

pathophysiology of EH because of anomalies in these functions. These results are in line with let-7a-5p's discoveries in another cardiovascular illness, where let-7a-5p and its target genes are connected to the control of the cell cycle and proliferation [19, 26, 27]. In addition, KEGG analysis identified p53 signaling pathway and FoxO signaling pathway as important signaling pathways involved. Previous studies have also demonstrated that miR-18 can potentially affect blood pressure through the P53 signaling pathway [28].

Taken together, let-7a-5p has the potential as an adjunctive diagnostic biomarker for EH. Based on correlation analysis and bioinformatics prediction, it is proposed that let-7a-5p may influence the pathogenesis of EH by regulating the RAAS system. This trial does have some limitations, nevertheless, as several of the conventional risk variables did not differ significantly between the two groups. This could be because the sample size was not balanced and was insufficient. At the same time, miRNA silencing experiments were not performed to clarify the value of let-7a-5p, and functional experiments were not performed to verify the mechanism of action of let-7a-5p, which led to the results remaining in the conjecture stage. Further expansion of the sample size, multicenter validation, as well as miRNA silencing experiments and functional experiments to validate the current conjecture are recommended for follow-up.

Author Contributions

Changwu Dong: Study conception and design, Funding Acquisition, and Resources. Lan Wang: Writing—Original Draft, Data collection, Investigation, Analysis. Qianqian Zhu: Investigation, Material Preparation, Literature Review, Data Curation. Bin Cheng: Writing—Review & Editing, Technical Support, Assistance with Experimental Design. Nan Jiang: Data Curation, Data Interpretation, Writing—Review & Editing. Approval of the final manuscript to be published. Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved).

Ethics Statement

Ethical approval for this study was obtained from the Second Affiliated Hospital of Anhui University of Traditional Chinese Medicine (Anhui Acupuncture and Moxibustion Hospital) under the approval number 2022-zj-02. All patients/subjects participating in this study were fully informed about the study and signed an informed consent form. We ensured that the study was conducted in compliance with ethical principles.

Consent

All patients in this study were fully informed about the purpose, methods, and possible risks of the study and voluntarily signed an informed consent form. We respect and protect the privacy and autonomy of patients and ensure that no personal information of patients will be disclosed during the study.

Conflicts of Interest

The authors declare that they have no interest associated with any organization or individual at the time of writing this paper. We have ensured that the writing and publication of this paper were not influenced by any external factors and that the objectivity and impartiality of the research were maintained.

Data Availability Statement

All data that support the findings of this study are included in this manuscript and its supporting information files.

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