Molecular Therapy
Nucleic Acids
Original Article

Circulating lncRNA BC030099 Increases in Preeclampsia Patients

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Long noncoding RNAs (lncRNAs) have increasingly been shown to be important biological regulators involved in numerous diseases. Further, increasing evidence demonstrates that circulating lncRNAs can be used as diagnostic biomarkers. Therefore, the purpose of this study was to evaluate the potential for circulating lncRNAs as novel biomarkers for the diagnosis of preeclampsia. In the present study, we measured the expression of five lncRNAs known to be relevant to the uterus in whole blood samples from 48 preeclampsia patients and 24 non-preeclampsia healthy subjects using qRT-PCR. We found that circulating levels of lncRNA BC030099 were significantly higher in patients with preeclampsia (1.232 ± 0.4870) than in non-preeclampsia healthy subjects (0.9928 ± 0.2008, $p < 0.05$). The area under the receiver operating characteristic (ROC) curve for lncRNA BC030099 was 0.713. Univariate and multivariate analyses identified lncRNA BC030099 as an independent predictor for preeclampsia. In brief, our results suggest that increased plasma levels of lncRNA BC030099 are associated with an increased risk of preeclampsia and may be considered a novel biomarker.

INTRODUCTION

Preeclampsia is a pregnancy-specific disease with multi-organ involvement and is characterized by new-onset hypertension with proteinuria after 20 weeks of gestation. Further, preeclampsia is a major cause of fetal and maternal death, affecting 3%–5% of pregnant women worldwide.[1,2] To date, there are no effective pharmacological agents for treating preeclampsia, and the effective solution is delivery or premature termination of the pregnancy. Although blood pressure and urinary protein excretion are the most commonly used clinical markers to diagnose preeclampsia,[3] these traditional methods are subject to nonspecific prediction and poor accuracy during the early stage of gestation. Without proper and timely prevention, preeclampsia may progress to a more severe stage known as eclampsia, which is accompanied by seizures and other severe impairments.[4,5] Early detection of preeclampsia with noninvasive and reliable biomarkers is the foremost step for minimizing adverse effects during pregnancy. The search for new preeclampsia biomarkers, particularly those for an early and precise diagnosis, is an urgent priority and has been the focus of a continuous effort from fundamental and clinical researchers worldwide. Phosphatidylinositol-glycan biosynthesis class F (PlGF), placental protein 13 (PP13), endothelin-1, plasma-P-selectin, and pregnancy-associated plasma protein A (PAPP-A) have been suggested as promising markers for preeclampsia.[6] In addition, recent studies have suggested the potential value of RNA biomarkers for preeclampsia.[7,8]

Long, non-coding RNAs (lncRNAs) include transcripts longer than 200 nt that cannot be translated into proteins, as they lack significant open reading frames or protein-coding capacity. Previously, lncRNAs were thought to represent transcriptional noise without any function; however, recent evidence suggests that lncRNAs are important regulators in chromatin imprinting and modification, genomic interference, and activation and inhibition of transcriptional regulatory networks.[8,9] In addition, lncRNAs are increasingly thought to be involved in diverse physiological and pathological processes such as cell proliferation, stem cell differentiation, epigenetic regulation, necrosis, and apoptosis.[10,11] He et al. screened lncRNA expression profiles in placentas from preeclampsia patients and healthy subjects and showed that 259 of 28,443 lncRNAs were upregulated and that 479 of 28,443 lncRNAs were downregulated in the placentas from preeclampsia patients,[12] suggesting that aberrant expression of lncRNAs may contribute to the pathogenesis of preeclampsia. Therefore, we aimed to identify potential lncRNAs as circulating biomarkers for preeclampsia.

RESULTS

Clinical Characteristics of the Study Population

Blood samples were collected from a total of 72 patients, including 24 non-preeclampsia healthy subjects and 48 patients with preeclampsia. Systolic (149.91 ± 23.50 mmHg versus 123.64 ± 19.01 mmHg) and diastolic blood pressure (98.21 ± 16.09 mmHg versus 82.82 ± 16.99 mmHg) were higher in the preeclampsia group than in the non-preeclampsia group. Further, there were statistically significant differences between urinary protein (PRO) detected in the preeclampsia

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Table 1. The Clinical Characteristics Assessed at Enrollment

Characteristic	Preeclampsia		p
	Yes (n = 48)	No (n = 24)	
Age (Years)			
n (missing)	47 (1)	22 (2)	0.454
Mean (SD)	30.77 (5.29)	29.82 (4.65)	
Median (IQR)	30 (27–34)	29 (28–32)	
Diabetes			
Yes	1 (2.1%)	0	1
No	46 (97.9%)	22 (100%)	
Total (missing)	47 (1)	22 (2)	
Blood Pressure (Systolic)			
Mean (SD)	149.91 (23.50)	123.64 (19.01)	<0.001
Median (IQR)	151 (130.5–165.5)	117 (112–134)	
Total (missing)	47 (1)	22 (2)	
Blood Pressure (Diastolic)			
Mean (SD)	98.21 (16.09)	82.82 (16.99)	<0.001
Median (IQR)	100 (88–111)	78.5 (74–96)	
Total (missing)	47 (1)	22 (2)	
Hypertension History			
Yes	0	0	
No	47	22	
Total (missing)	47 (1)	22 (2)	
Smoking History			
Yes	0	0	
No	47	22	
Total (missing)	47 (1)	22 (2)	
CHOL			
Mean (SD)	6.59 (1.58)	6.75 (1.53)	0.807
Median (IQR)	6.430 (5.655–7.115)	6.33 (6.12–7.61)	
Total (missing)	15 (33)	9 (15)	
TG			
Mean (SD)	3.56 (1.29)	4.40 (1.58)	0.198
Median (IQR)	3.34 (2.78–3.975)	4.25 (3.28–5.19)	
Total (missing)	15 (33)	9 (15)	
HDL			
Mean (SD)	1.90 (0.40)	1.55 (0.34)	0.063
Median (IQR)	1.79 (1.65–1.92)	1.640 (1.325–1.780)	
Total (missing)	14 (34)	7 (17)	
LDL			
Mean (SD)	3.45 (0.97)	3.6 (1.27)	0.790
Median (IQR)	3.42 (2.52–3.89)	3.790 (3.045–4.500)	
Total (missing)	14 (34)	7 (17)	

Table 1. *Continued*

Characteristic	Preeclampsia		p
	Yes (n = 48)	No (n = 24)	
Hypersensitive C Protein			
Mean (SD)	5.48 (5.26)	8.34 (5.33)	0.348
Median (IQR)	3.360 (1.645–8.980)	6.58 (3.90–13.66)	
Total (missing)	11 (37)	5 (19)	
Blood Glucose			
Mean (SD)	4.85 (1.08)	4.93 (1.73)	0.830
Median (IQR)	4.600 (4.100–5.265)	4.49 (3.91–4.85)	
Total (missing)	47 (1)	21 (3)	
CHD			
Yes	1	0	1
No	46	23	
Total (missing)	47 (1)	23 (1)	
Gestational Age (Weeks)			
Mean (SD)	34.45 (4.68)	37.64 (3.08)	0.001
Median (IQR)	35.14 (30.57–38.855)	38.64 (34.57–40.00)	
Total (missing)	47 (1)	22 (2)	
Glycosuria			
Mean (SD)	0.152 (0.363)	0.272 (0.55)	0.36
Median (IQR)	0 (0)	0 (0)	
Total (missing)	46 (2)	22 (2)	
Proteinuria			
Mean (SD)	2.30 (1.47)	0.91 (1.11)	<0.001
Median (IQR)	3 (1–3)	1 (0–1)	
Total (missing)	46 (2)	22 (2)	
Specific Gravity			
Mean (SD)	1.024 (0.012)	1.02 (0.009)	0.035
Median (IQR)	1.022 (1.013–1.033)	1.0175 (1.01–1.024)	
Total (missing)	46 (2)	22 (2)	
Fetal Heartbeat			
Mean (SD)	143.09 (8.71)	145.63 (4..24)	0.11
Median (IQR)	145 (140–150)	145 (142–150)	
Total (missing)	45 (3)	22 (2)	
Edema (N)			
0	20 (0.435)	17 (0.773)	
1	13 (0.283)	4 (0.182)	
2	6 (0.130)	0	0.1
3	5 (0.1090)	1 (0.045)	
4	2 (0.043)	0	
Total (missing)	46 (2)	22 (2)	

(*Continued on next page*)

Table 1. *Continued*

Characteristic	Preeclampsia		p
	Yes (n = 48)	No (n = 24)	
Placental Function (N)			
0	2 (0.043)	0	0.63
1	18 (0.383)	7 (0.318)	
2	21 (0.447)	10 (0.455)	
3	6 (0.128)	5 (0.227)	
Total (missing)	47 (1)	22 (2)	
Fetal Maturity			
Mean (SD)	32.76 (4.73)	35.45 (5.24)	0.07
Median (IQR)	33.43 (28.29–36.71)	37.29 (33.71–39.00)	
Total (missing)	45 (3)	22 (2)	

CHOL, total cholesterol; TG, triglyceride; HDL, high-density cholesterol; IQR, interquartile range; LDL, low-density cholesterol; CHD, coronary heart disease.

patients and that detected in the non-preeclampsia control subjects (2.30 ± 1.47 versus 0.91 ± 1.11 g/24 h, respectively). Additionally, preeclampsia patients had an obviously shorter gestational period than non-preeclampsia patients did (34.45 ± 4.68 versus 37.64 ± 3.08 weeks, respectively). There were no significant differences in age, diabetes, coronary heart disease, cholesterol (CHOL), triglycerides (TGs), high-density lipoproteins (HDLs), or low-density lipoproteins (LDLs) between the preeclampsia and non-preeclampsia groups (Table 1).

Expression Levels of Five lncRNAs in the Whole Blood of Patients with Preeclampsia

lncRNA expression profiles had shown that a cluster of lncRNAs was upregulated in the placentas of patients with preeclampsia and that GenBank: NR_026824.1, AK055151.1, NR_027457, NR_024178, and BC030099 were five of them.[12] We used qRT-PCR analyses to determine expression levels of these five lncRNAs in the whole blood (WB) from patients with preeclampsia. However, as illustrated in Figures 1A–1E, only lncRNA BC030099 levels in the WB samples were significantly different between the preeclampsia and non-preeclampsia groups. As such, circulating levels of BC030099 were significantly higher (24%) in patients with preeclampsia (1.232 ± 0.4870) than in non-preeclampsia subjects (0.9928 ± 0.2008, $p < 0.05$). The median cycle threshold (Ct) value for lncRNA BC030099 was 24.64, ranging from 23.14 to 26.47. These results indicate that lncRNA BC030099 is stable and abundant in human blood.

Relationship between Circulating lncRNA BC030099 and Preeclampsia

We used receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) to confirm the relationship between lncRNA BC030099 and preeclampsia. The ROC curve for BC030099 was 0.713 (95% confidence interval [CI] = 0.587–0.839), while the ROC curves for the other 4 lncRNAs tested (NR_026824.1, AK055151.1, NR_027457, and NR_024178) were 0.594 (95% CI = 0.462–0.726), 0.512 (95% CI = 0.372–0.652), 0.532 (95% CI = 0.387–0.676), and 0.542 (95% CI = 0.407–0.677), respectively, which were not as good as that measured for lncRNA BC030099 (Figure 2).

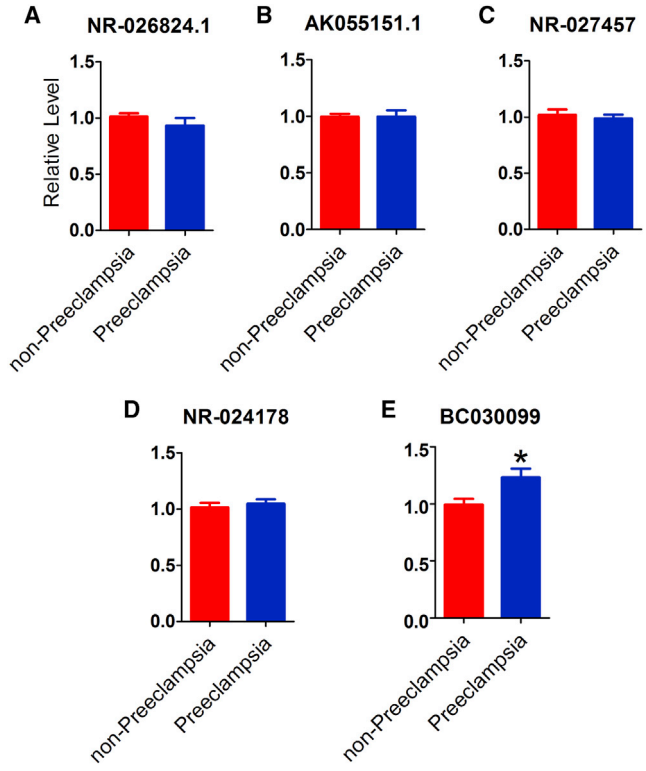

Figure 1. Expression of circulating long non-coding RNAs (lncRNAs) in the peripheral blood of patients with preeclampsia or non- preeclampsia

(A–E) Circulating levels of lncRNAs were determined via real-time qRT-PCR with plasma samples prepared from preeclampsia patients and non-preeclampsia control participants. (A) NR_026824.1. (B) AK055151.1. (C) NR_027457. (D) NR_024178. (E) BC030099. Significant differences between patients with preeclampsia and non-preeclampsia control participants were only observed for lncRNA BC030099. Data are presented as means ± SEM. *$p < 0.05$; N = 48 for preeclampsia, and N = 24 for non-preeclampsia control participants.

DISCUSSION

Symptoms of preeclampsia, a systemic complication of pregnancy, include hypertension, proteinuria, and pathologic edema. Preeclampsia is a major cause of maternal and fetal death. Diagnostic laboratory results include hemolysis, elevated liver enzymes, and decreased platelet count (HELLP syndrome). The diagnostic criteria for preeclampsia include pregnancy after 20 weeks, a systolic blood pressure greater than 140 mmHg, a diastolic blood pressure greater than 90 mmHg, and proteinuria higher than 0.3 g/24 h. To date, the only means for treating preeclampsia is the induction of delivery. Immune maladaptation, inadequate placental development, and placental ischemia are all thought to be key factors in the development of preeclampsia. The pathogenesis of preeclampsia is complex; therefore, treatment, as well as early and accurate prediction, is necessary to prevent preeclampsia deteriorating into eclampsia. Therefore,

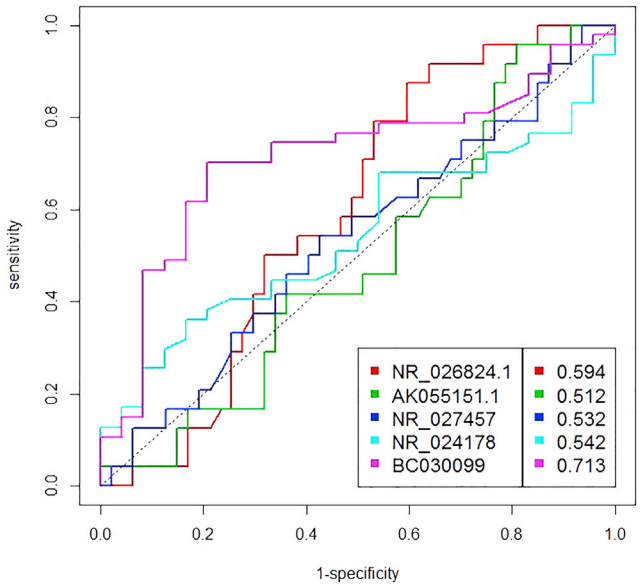

Figure 2. ROC Analysis of Circulating lncRNAs for Predicting Preeclampsia
The area under the ROC curve was determined to evaluate the predictive power of circulating lncRNA- NR_026824.1, AK055151.1, NR_027457, NR_024178, and BC030099 levels for preeclampsia using non-preeclampsia participants as controls.

urgent efforts are required to find and identify effective circulating markers for accurate prediction and effective treatment.

lncRNAs are a newly discovered class of gene expression regulators. Recently, lncRNAs have been attracting the attention of researchers worldwide, as they have been shown to participate in a wide spectrum of biological processes and have been characterized as potential biomarkers for human disease. Research by Luo et al. provided a series of lncRNAs, including NR_027457, AF085938, G36948, and AK002210, that serve as potential diagnostic biomarkers for preeclampsia and have paved the way for the use lncRNAs as biomarkers for preeclampsia.[7]

Previous studies confirmed that NR_026824.1, AK055151.1, NR_027457, NR_024178, and BC030099 levels are higher in placentas from preeclampsia patients. The findings of this study confirm that these five lncRNAs participate in the process of preeclampsia. However, it remains unknown whether these preeclampsia-related lncRNAs can be used as novel biomarkers for preeclampsia. Therefore, the aim of this study was to identify potential circulating biomarkers for detecting preeclampsia at an early stage.

Although expression levels of NR_026824.1, AK055151.1, NR_027457, and NR_024178 were extremely high in the placenta from preeclampsia patients, there were no obvious changes in circulating levels between preeclampsia patients and non-preeclampsia patients. Moreover, the AUCs for these four lncRNAs were less than 0.6, indicating that they are not suitable as biomarkers for preeclampsia. However, expression levels of BC030099 were significantly higher in blood from preeclampsia patients than those from non-preeclampsia subjects. Further, statistical analyses showed that the lncRNA BC030099 correlates well with preeclampsia, and the AUC for the lncRNA BC030099 was 0.713 according to the ROC curves. These findings indicate that circulating lncRNA BC030099 is a potential predictor of preeclampsia with significant potential.

The discovery of deregulated BC030099 opens new opportunities for using this lncRNA as a diagnostic marker. In addition, these findings shed light on a new layer involved in the regulatory network of human disease and, especially, preeclampsia therapeutic targets.

MATERIALS AND METHODS

Participants

Circulating blood samples from 48 preeclampsia patients were collected between 2015 and 2017 from the Second Affiliated Hospital of Harbin Medical University (Harbin, China). Blood samples from 24 non-preeclampsia subjects were also obtained from healthy pregnant women. The healthy pregnant women were recruited as control subjects at the time of a regular medical checkup. The blood samples from both groups were collected at 30–40 weeks after pregnancy. Clinical characteristics assessed at enrollment are shown in Table 1. All patients and control subjects included in our study belong to the Han nationality.

Ethical Approval of Studies and Informed Consent

All experimental protocols were approved by the Harbin Medical University ethics committee for use of human samples, and the methods were carried out in accordance with the approved guidelines. All procedures involving human subjects were approved by the Institutional Research Board of Harbin Medical University.

Table 2. The Primer Sequences of the Related lncRNAs

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
NR_026824.1	CTATGGTCCAGACCTCCCC	TTCCCATTAATTGTCCAAGAGTAC
AK055151.1	CCTGCTTCGCGGAATGTAA	GCTTTGTAGTAATTTGGGGGATAA
NR_027457	TCTGGAGAAGGAGAGTTGGC	TCCTCACATGCCCCATTTCT
NR_024178	ACAGGCTAGGAGAGAAGGGA	CTTAAGCATCTTCACGGCCC
BC030099	CAGCTGAGCGGGACATTTAC	GGTCTGAAAGGATGGAGGCT

Collection and Handling of Human Blood Samples

WB samples (1 mL per patient) were drawn from the study subjects via direct venous puncture into tubes containing sodium citrate for lncRNA detection. The blood samples were stored at −80°C until use.

qRT-PCR

Total RNA was isolated from 1 mL WB using a phenol-chloroform extraction procedure, and qRT-PCR was performed as previously described.[13] The PCR primer pairs are listed in Table 2.

Statistical Analysis

Quantitative data are described as means ± standard deviation error of the mean (SDEM), median, and interquartile. Qualitative data were described as count and percentage. Differences between two groups for quantitative data and qualitative data were compared using a t test and chi square test, respectively. All analyses were performed using SPSS 13.0, and the significance level was set at 0.05.

AUTHOR CONTRIBUTIONS

Y.S. conceived and designed all experiments. Y.H. is responsible for all of the statistics. N. Lv, Q.L., N. Lin, and S.Z. are in charge of the blood collection. X. Chu, X. Chen, and G.C. conducted qRT-PCR. P.L. checked the data and wrote this paper.

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