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Original Article

Downregulation of lncRNA-ATB involved in preterm birth

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Abstract: LncRNA-ATB is involved in the pathogenesis of various types of diseases including preeclampsia, while its involvement in preterm birth is unknown. In this study, expression of lncRNA-ATB in placenta and serum of both females who gave preterm birth (n=108) or full term birth (n=99) was detected by qRT-PCR. Diagnostic values of lncRNA-ATB for preterm birth were analyzed by ROC curve analysis. Correlations between lncRNA-ATB expression levels and clinicopathological data of females who gave preterm birth were analyzed by Chi-square test. Another 320 pregnant women were included and divided into high and low expression group according to the median serum level of lncRNA-ATB, and occurrence of preterm birth was recorded and compared between 2 groups. We found that lncRNA-ATB was downregulated in females who gave preterm birth comparing to those who gave full term birth. LncRNA-ATB expression levels could be used to effectively distinguish females who gave preterm birth from those who gave full term birth. Expression levels of lncRNA-ATB were significantly associated with patients' age, but not other general data. Patients with higher serum level of lncRNA-ATB showed lower incidence of preterm birth. Therefore, downregulation of lncRNA-ATB is likely involved in preterm birth.

Keywords: Premature birth, RNA, long noncoding, pathology, molecular

Introduction

As a type of birth occurring before 37 weeks of gestation, preterm birth is one of the leading causes of death in children younger than 5 years old worldwide [1]. It has been reported that more than 10% infants in the United State are born preterm [2]. In China, preterm birth occurs in 7.1% of all deliveries and neonatal mortality rate among live preterm births is about 3.3% [3]. Mortality rate of infants and incidences of serious acute morbidities including necrotizing enterocolitis, respiratory distress syndrome and intraventricular hemorrhage are inversely correlated with gestational age [4]. Early diagnosis of preterm birth plays a pivotal role in delaying gestational age [5], while sensitive diagnostic markers remain lacking.

Genetic factors play pivotal roles in the pathogenesis of preterm birth [6]. Recent studies have shown that pregnant women gave preterm birth shows altered expression patterns of a large set of lncRNAs compared with those who gave full term birth, indicating the possible involvement of lncRNAs in this disease [7]. LncRNA-ATB is characterized as an oncogene in the pathogenesis of different types of human malignancies [8, 9]. A recent study showed that downregulation of lncRNA-ATB is involved in preeclampsia [10]. In this study, it was found that downregulation of ATB expression may serve as a potential diagnostic biomarker for preterm birth.

Materials and methods

Patients

Our study included 108 females who gave preterm birth (patient group) and 99 females who gave full term birth (control group). Those pregnant women were admitted to Haidian Maternal & Child Health Hospital from January 2015 to January 2018. Inclusion criteria: 1) singleton birth; 2) gave birth for the first time; 3) received no treatment before admission; 4) at least 20 years old; 5) willing to participate in this study. Exclusion criteria: 1) with other pregnancy complications; 2) with serve malignancies. Age of patient group ranged from 21 to 45 years old,

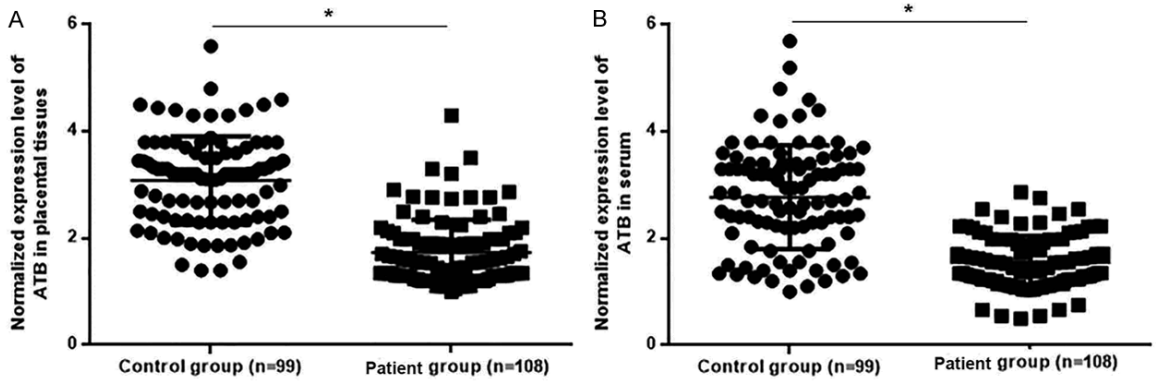

Figure 1. Comparison of expression levels of ATB in placental tissues and serum between patient group and control group. Comparison of expression levels of ATB in placental tissues (A) and serum (B) between patient group and control group. Compared with control group, expression of ATB was significantly downregulated in patient group. *, $p<0.05$.

with a mean age of 32.4±3.2 years. Age of control group ranged from 20 to 44 years, with a mean age of 32.7±3.5 years. No significant differences in age and other basic data were found between two groups. Placental tissues (100-150 mg, collected through transabdominal placental biopsy) and blood (5 ml) were extracted in a morning of 26 gestational weeks and serum was prepared. Besides that, another 320 pregnant women were also included according to the same inclusion and exclusion criteria. Blood (5 ml) was extracted from those participants in a morning of 13 gestational weeks, which is used for the study of the correlation between expression level of ATB and preterm birth, to prepare serum. This study was approved by the ethics committee of Haidian Maternal & Child Health Hospital. All patients signed informed consent.

Real-time quantitative PCR (qRT-PCR)

All total RNA extractions were performed using Trizol reagent (Invitrogen, USA). Placental tissues were ground in liquid nitrogen before adding Trizol reagent. Those RNA samples were subjected to reverse transcription to synthesize cDNA using SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific) according to the following conditions: 50°C for 15 min and 85°C for 20 min. PCR reaction systems were prepared using SYBR® Green Real-Time PCR Master Mixes. Sequences of primers were: 5'-CTTCACCAGCACCCAGAGA-3' (forward) and 5'-AAGACAGAAAAACAGTTCCGAGTC-3' (reverse) for lncRNA ATB; 5'-GACCTCTATGCCAACACAGT-3' (forward) and 5'-AGTACTTGCGCTCAGGAGGA-3' (reverse) for human β-actin. Conditions of PCR reactions were: 1 min at 95°C, followed by 40 cycles of 20 s at 95°C and 50 s at 55°C. $2^{-\Delta\Delta CT}$ method was used to process all Ct values and normalize ATB expression to β-actin.

Statistical analysis

Graphpad Prism 6 software was used to process all data. ATB expression data were expressed as ($\bar{x}$ ± SD) and compared by unpaired t test between two groups of participants. Chi-square test was used to analyze the correlations between ATB expression and patients' basic information, including age, pre-pregnancy BMI, and smoking and drinking habits. ROC curve analysis was performed using normalized ATB expression of both patient group and control group with default parameters. $p<0.05$ indicates a difference with statistical significance.

Results

Expression levels of ATB in placental tissues and serum are lower in patients with preterm birth than in control group

Differential expression of certain genes in patients and healthy controls usually indicates the involvement of this gene in this disease. We therefore measured the expression levels of placental tissues and serum in both patient group and control group. As shown in **Figure 1A**, expression levels of lncRNA ATB in placen-

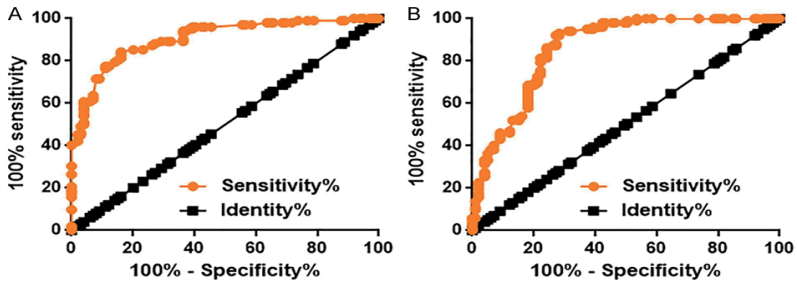

Figure 2. Diagnostic values of ATB expression for preterm birth. The diagnostic values of ATB expression in placental tissue (A) and serum (B) for preterm birth. Downregulation of ATB expression effectively distinguish patient group from control group.

tal tissues were significantly lower in patient group than in control group ($p<0.05$). In addition, serum levels of lncRNA ATB were also significantly lower in patient group than in control group (**Figure 1B**) ($p<0.05$). Therefore, downregulation of lncRNA ATB is likely involved in the pathogenesis of preterm birth.

Downregulation of ATB distinguished preterm birth patients from healthy controls

ROC curve analysis was performed to evaluate the diagnostic values of ATB expression in placental tissues and serum for preterm birth (**Figure 2**). Regarding ATB expression in placental tissues, the area under the curve (AUC) was 0.9046 (standard error: 0.02079; 95% confidence interval: 0.8639 to 0.9454). As for serum ATB, AUC was 0.8607 (standard error: 0.02647; 95% confidence interval: 0.8088 to 0.9126). Therefore, ATB expression may serve as a potential diagnostic marker for preterm birth.

ATB expression levels in placental tissues were significantly correlated with patients' age and smoking habit

Chi-square test was performed to analyze the correlations between ATB expression and patients' basic data. As shown in **Table 1**, levels of ATB expression in placental tissues were significantly correlated with patients' age and smoking habit ($p<0.05$), but not pre-pregnancy BMI and drinking habit ($p>0.05$). In addition (**Table 2**), serum levels of ATB were only significantly correlated with patients' smoking habit ($p<0.05$).

Low serum level of ATB predicted high risk of preterm birth

According to the median serum level of ATB, the 320 pregnant women were divided into high (n=160) and low (n=160) expression groups. Preterm birth occurred in 24 cases of low expression group (n=160), and the incidence rate was 15%. In contrast, preterm birth occurred in 10 cases of high expression group (n=160), and the incidence rate was 6.25%. Therefore, incidence of preterm birth in low expression group was obviously higher than that in high expression group. Among the 34 cases of preterm birth, 20 cases occurred in 28-32 gestational weeks (early preterm birth, group A) and 14 cases occurred in 33-37 gestational weeks (late preterm birth, group B). As shown in **Figure 3**, serum level of ATB in group A were significantly lower than that in group B.

Discussion

The key finding of our study is that as an oncogenic lncRNA ATB plays pivotal roles in the pathogenesis of different types of human malignancies and preeclampsia [8-10] is also likely involved in preterm birth. Detection of serum ATB during early pregnancy stage (13 gestational weeks in this study) has predictive value for preterm birth.

As an oncogenic lncRNA, ATB is overexpressed in many types of human malignancies and promote cancer cell proliferation, migration and invasion [8, 9]. In contrast, downregulation of ATB has been observed in preeclampsia, and reduced expression level of ATB results in inhibited migration, proliferation, and tube formation of trophoblast cells [10]. It has been reported that the occurrence of preterm birth also requires the involvement of multiple lncRNAs [11]. Our preliminary microarray data showed that lncRNA ATB was downregulated in placental tissues derived from females who gave preterm births than in those derived from females who gave full term births (data not shown). Our further qRT-PCR experiments con-

Table 1. Correlations between ATB expression in placental tissues and patients' basic information

Variables	Groups	Cases	High-expression	Low-expression	χ^2	*p* value
Age	>35 (years)	28	8	20	7.66	0.02
	25-30 (years)	45	24	21		
	<25 (years)	35	22	13		
Pre-pregnancy BMI	<18.5	29	13	16	0.93	0.63
	18.5-23	55	30	25		
	>23	24	11	13		
Smoking	Yes	30	10	20	4.62	0.03
	No	78	44	34		
Drinking	Yes	39	17	22	1.00	0.32
	No	69	37	32		

Table 2. Correlations between serum levels of ATB and patients' basic information

Variables	Groups	Cases	High-expression	Low-expression	χ^2	*p* value
Age	>35 (years)	28	9	19	5.17	0.08
	25-30 (years)	45	24	21		
	<25 (years)	35	21	14		
Pre-pregnancy BMI	<18.5	29	12	17	4.56	0.10
	18.5-23	55	33	22		
	>23	24	9	15		
Smoking	Yes	30	9	21	6.65	0.01
	No	78	45	33		

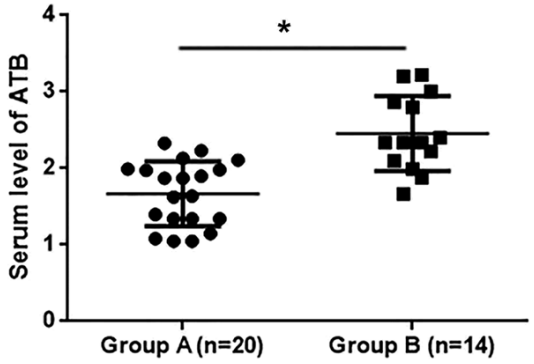

Figure 3. Comparison of serum levels of ATB between patients with early and late preterm birth. *, $p<0.05$.

firmed the downregulated expression of lncRNA ATB in females who gave preterm births than in those who gave full term births in both placental tissues and serum. It is suggested that downregulation of lncRNA ATB is related to the occurrence of preterm birth.

With the advantage of non-invasive nature, circulating biomarkers have been widely used in the diagnosis of human diseases [12, 13]. In this study, circulating lncRNA ATB was been detected in serum of all participants in patient and control groups. ROC curve analysis confirmed that downregulation of lncRNA ATB in both placental biopsies and serum could be used to effectively distinguish preterm birth-given females from full term birth-given females. We also observed that the performance of lncRNA ATB expression in placental biopsies is slightly better than serum circulating ATB. However, serum ATB detection as a non-invasive technique may be used to assist the diagnosis of preterm birth in cases of the invasive placental biopsy is not applicable.

Occurrence of preterm birth has been proved to be correlated with various factors. Smoking has long been recognized as an independent risk factor for preterm birth [14], and reducing tobacco smoking and smoke exposure contributes to the extension of gestational age [15]. Advanced maternal age also contributes to the occurrence of preterm birth [16, 17]. Females at the extremes of pre-pregnancy BMI are at risk for preterm birth [18]. In contrast, the contribution of alcohol consumption is still controversial [14, 19]. In this study, expression

levels of ATB in both placental biopsies and serum were significantly correlated with patients' smoking habit. Therefore, ATB may participate in preterm birth through smoking dependent pathways.

Early diagnosis of preterm birth is the key for its prevention [20]. Our study confirmed that detection of serum ATB during early pregnancy stage (13 gestational weeks in this study) has predictive value for preterm birth. However, future studies with bigger sample size are still needed to further confirm the conclusions.

In conclusion, lncRNA-ATB is downregulated in females who gave preterm birth comparing to those who gave full term birth. Downregulation of ATB can be used to distinguish preterm birth-given females from full term birth-given females. Serum levels of ATB during early pregnancy stage (13 gestational weeks in this study) have predictive value for preterm birth.

Disclosure of conflict of interest

None.

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