

SCH58261, the antagonist of adenosine A2A receptor, alleviates cadmium-induced preeclampsia *via* sirtuin-1/hypoxia-inducible factor-1 α pathway in rats

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Abstract. – OBJECTIVE: To identify the role of adenosine A2A receptor (A2AR) in cadmium-induced preeclampsia (PE) rats and the potential molecular mechanism.

PATIENTS AND METHODS: The expression of A2AR in placentae obtained from PE women and normal pregnant (NP) women were measured. The pregnant rats were randomly divided into four groups, including NP rats, PE rats, SCH+NP rats, and SCH+PE rats. The 0.125 mg/kg/d CdCl₂ was used to establish a PE rat model in PE and SCH+PE rats. SCH58261 was used as the specific antagonist of A2AR with a concentration of 0.2 mg/kg in SCH+NP and SCH+PE rats. The conditions of mother, foetus, and placenta were tested. The placental expression of A2AR, sirtuin-1 (sirt1), and Hypoxia-Inducible Factor-1 α (HIF-1 α) was measured by Western blot (WB) and immunohistochemistry (IHC) staining.

RESULTS: A2AR and HIF-1 α increased, and sirt1 decreased in placenta in both PE women and cadmium-induced PE rats. After treatment with SCH58261, the sirt1 increased and HIF-1 α decreased in cadmium-induced PE rats along with the amelioration of maternal outcomes, foetal and placental growth.

CONCLUSIONS: This paper firstly revealed that placental A2AR mediated cadmium-induced PE, and A2AR suppression could attenuate placental impairment by acting on the expression of sirt1 and sirt1-mediated regulation of HIF-1 α .

Key Words:

Preeclampsia, Adenosine A2A receptor, Sirtuin-1, Hypoxia-inducible factor-1 α .

Introduction

Preeclampsia (PE) is a syndrome limited to pregnancy that presents with the hallmark clinical manifestations of new-onset hypertension and proteinuria after the 20th gestational week¹, which could increase considerably the risk of foetal growth restriction (FGR)². This disease is implicated in 2% to 8% of pregnancies and is considered as a common trigger for maternal and perinatal morbidity and mortality worldwide³. Placenta, a multifunctional organ, plays a central conciliatory role during pregnancy, whose proper structure and function are integral for a successful and smooth pregnancy⁴. Increasing evidence supports the view that the PE is primarily a placental dysfunctional disease^{5,6}.

Cadmium, a nonbiological heavy metal, exists ubiquitous in the environment, especially in the plant roots, such as rice, tobacco, etc. The population intakes cadmium via diet and smoking. Pregnant women are more susceptible to it due to the increased intestine absorbency and respiratory rate⁷. Placenta is recognized as the major organ target of cadmium. Besides, epidemiologic study has suggested that gestational cadmium exposure contributes to the development of PE although the mechanism remains mysterious.

The available data have shown that cadmium exposure during pregnancy links with the abnormal increase of adenosine^{8,9}. The proposed

mechanism of PE has included adenosine-dependent biological effects, adenosine receptors, and the associated adenosine signalling pathway¹⁰⁻¹³. Adenosine usually exhibits its effects by the attachment of specific receptors, such as A₁R, A_{2A}R, A_{2B}R, and A₃R¹⁴, whose activation has been distinguished as danger signals for placental maldevelopment^{11,15,16}. Among them, A_{2A}R is one type of high-affinity adenosine receptors, playing as an alarm sensor to tissue damage. As expected, the elevation of placental A_{2A}R occurs in PE patients¹⁷, and is exaggerated when complicated by FGR^{17,18}. Besides, this receptor is strongly positively correlated with the expression of HIF-1 α in human placenta throughout pregnancy with or without PE, and proved to be able to act with HIF-1 α to sustain tissue damage^{16,19}.

Although the enhancing effect of cadmium in trophoblast HIF-1 α expression in the development of PE has also been well recognized in previous studies²⁰⁻²³, the role of A_{2A}R in cadmium-related PE remains unknown. Thus, the purpose of the present work is to identify the effect of cadmium on the expression of A_{2A}R in placenta and address the role of receptor in cadmium-induced preeclamptic rats.

Patients and Methods

Patients

According to ACOG, PE was diagnosed by the new onset of hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg), and proteinuria (≥ 300 mg/24 h or random urine protein ≥ 0 (+)) after the 20th gestational week²⁴. The blood pressure and urine protein levels of all recruited volunteers recovered to normal levels within six weeks after delivery. The inclusive criteria of participants in this paper were from singleton pregnancies at term, the age of the pregnancy women was between 26 and 28. The exclusive criteria is that those pregnant women suffering from cardiovascular disease, kidney disease, or other complications of pregnancies were excluded. Normotensive pregnant women were randomly employed as controls. Normotensive pregnant women (n=5) and pregnant patients with PE (n=5) were included in the study. All protocols applied in our research were approved by the Clinical Research Committee of The First Affiliated Hospital of Wenzhou Medical University.

Rats and Treatments

Adult female SD rats (220 \pm 10 g) purchased from the Animal Centre of Chongqing Medical University were maintained with a standard chow diet and standard 12:12 h light-dark cycle at a controlled temperature of 25°C. Female rats were mated with male rats overnight. The day on which sperm was identified from the vaginal smear was defined as gestational day 0 (GD0). The pregnant animals were randomly assigned to the following four groups containing five rats each, the normal pregnancy (NP) rats, normal pregnancy with SCH58261 (SCH+NP) rats, preeclampsia (PE) rats, and preeclampsia with SCH58261 (SCH+PE) rats. From GD9 to GD18, SCH groups (SCH+NP and SCH+PE rats) were intraperitoneally injected with SCH58261 (Beyotime, Shanghai, China) at a concentration of 0.2 mg/kg/d at 7:30, and the rest of the rats were treated with saline at an equivalent volume at the same time. From GD9 to GD14, rats in the CdCl₂-treated groups (PE and SCH+PE rats) were intraperitoneally injected with CdCl₂ (0.125 mg/kg/d, obtained from Sinopharm Chemical Reagent Co., Ltd., Mainland, China) at 8:00; rats in the other groups were treated with saline at an equivalent volume at the same time. The dosage of SCH58261 used was determined based on the literature²⁵. All procedures used in this study complied with the Institutional Animal Care and Use Committee (IACUC).

Measurement of Systolic Blood Pressure (SBP)

All female rats were trained to adapt to blood pressure measurement before the start of the experiment. SBP was measured between 8:00 and 12:00 every three days from GD3 to GD18 with a noninvasive pressure monitor (BP-98A, Softron Co., Ltd., Beijing, China). The average value from three consecutive SBP readings (that differed by no more than six mmHg) was viewed as the SBP value for that day.

Urine Protein Quantitation

On GD3 and GD18, the rats were fasted in metabolic cages with water for 24 h. The urine was collected, and protein was quantified by the bicinchoninic acid assay (BCA) method purchased from Thermo Fisher Scientific, Waltham, MA, USA).

Sample Collection

Rat pups were delivered on GD19 by cesarean section after anesthetization with an intraperitoneal injection of 1% pentobarbital. One kidney

and three placentae were randomly chosen from each maternal rat and processed with 4% paraformaldehyde for histological evaluation. The remaining placentae were kept at -80°C . Fetal weight, fetal length, placental diameter, and placental weight were measured. Placental efficiency was defined by fetal weight/placental weight²⁶. The FGR rate was defined as the proportion of foetuses born below the 10th weight percentile for the gestational age²⁷.

Morphological Assay and Immunohistochemistry (IHC) Staining

Paraffin slices (4- μm thick) of the kidney and placentae were processed by HE staining for microstructural observation. The 4- μm thick placental slices were stained with anti-A_{2A}R, anti-sirt1, and anti-HIF-1 α , respectively, in a wet darkroom at 4°C overnight, followed by an anti-rabbit/mouse auto-immunostaining (ZSGB-BIO, Beijing, China). The pictures were taken under a light microscope (Nikon, Shanghai, China), and analysed by ImageJ. All primary antibodies used were from Abcam (Shanghai, China).

TUNEL Staining

Placental apoptosis was detected with a TUNEL kit (Roche, Shanghai, China). Apoptotic nuclei were identified by brown staining, and the condensed heterochromatic nuclei in 5 random high-power fields were counted. The apoptotic index was defined as the number of apoptotic nuclei compared to the total number of nuclei²⁸. Two independent and blinded counters were employed, and the data were averaged.

Western Blot (WB)

The total protein was extracted from placental tissue with radio immunoprecipitation assay (RIPA) buffer (Active Motif Company, Carlsbad, CA, USA) and quantified by the bicinchoninic acid assay (BCA) method. Then, the proteins (40 μg) were separated in 8% Tris-glycine mini-gels, followed by transfer to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated in 5% bovine serum albumin (BSA) in Tris-Buffered Saline and Tween-20 (TBST) for 90 min and incubated with primary antibodies (anti- β -actin, anti-sirt1, anti-A_{2A}R, and anti-HIF-1 α) at 4°C overnight (all were from Abcam, Shanghai, China). The next day, the membranes were washed in Tris-Buffered Saline and Tween-20 (TBST) three times and probed with horseradish peroxidase (HRP)-conjugated species-specific second-

ary antibody (the secondary antibodies were from Sangon, Shanghai, China) for 60 min. Finally, the bands were developed with an enhanced chemiluminescence (ECL) solution (Pierce, Rockford, IL, USA) in a Bio-Rad ChemiDoc XRS system (USA), and the relative bands densities were evaluated with AlphaEaseFC software.

RT-qPCR

Total RNA was extracted from placental tissues with RNAiso Plus solution following the manufacturer's instructions. The RNA was reverse transcribed into cDNA with a PrimeScript RT Reagent Kit and then amplified with TB Green[®] Premix Ex Taq[™] II. The comparative cycle threshold method was employed for the analysis of relative gene expression against β -actin. All reagent kits used in the experiments described above were provided by TaKaRa (Dalian, China). The following primers were used in our study: sirt1 forward (TGG CAC CGA TCC TCG AAC), sirt1 reverse (CCG CTT TGG TGG TTC TGA AAG), β -actin forward (TGT CAC CAA CTG GGA CGA TA), and β -actin reverse (GGG GTG TTG AAG GTC TCA AA), HIF-1 α forward (AGT TAC CTG CCC TGT CCT CT), HIF-1 α reverse (GTA GCA GTG GCT GTG GTT CT).

Statistical Analysis

The results were presented as the mean \pm SD. Comparisons of two groups were assessed by Student's *t*-test. One-way ANOVA was performed to test the difference between three or more groups. Frequency data were performed by the chi-square test. SPSS software (IBM, Armonk, NY, USA) was employed for analyses, a significant difference was concluded when $p < 0.05$.

Results

The Placental A_{2A}R Expression in PE Women and Cadmium-Induced Preeclamptic Rats

The placentae from PE patients and cadmium-induced PE rats were obtained for IHC staining and WB to identify the expression of A_{2A}R. As seen in Figure 1A-D, A_{2A}R increased in human placenta in the results of IHC staining (Figure 1A and B, $p < 0.01$) and WB (Figure 1C and D, $p < 0.05$). The consistent tendency of A_{2A}R in cadmium-induced PE rats was detected by the IHC staining (Figure 1E and F, $p < 0.01$) and WB (Figure 1G and H, $p < 0.05$).

The Effect of SCH58261 on the Maternal Outcome in Cadmium-Induced PE Rats

Following the identification of the consistent abnormal increase of placental A_{2A} R in PE women and cadmium-induced PE rats, SCH58261 was used to test the effect of A_{2A} R in the cadmium-induced PE rats. Before drug treatment, there was no difference in indicators among groups. After cadmium treatment, cadmium significantly decreased maternal body weight (Figure 2A, $p < 0.05$), increased SBP (Figure 2B, $p < 0.01$) in the PE rats beginning at GD12, and aggravated proteinuria (Figure 2C, $p < 0.01$) compared to NP rats. When cadmium combined with SCH58261 treatment, the maternal weight was improved beginning at GD15 (Figure 2A, $p < 0.05$), SBP was decreased beginning at GD18 (Figure 2B, $p < 0.05$), and proteinuria was attenuated as well (Figure 2C, $p < 0.01$) in the SCH+PE rats in contrast to the PE rats. However, the above indexes were not significantly different between the NP and SCH+NP rats (Figure 2bA-C, $p > 0.05$). The observation of kidney changes (Figure 2D) suggested that the glomerular structure was normal in the NP and SCH+NP rats, and the abnormalities of endothelial hyperplasia and narrowing of the capillary vessels were shown in PE rats, which could be attenuated by SCH58261 combined treatment.

The Effect of SCH58261 on Foetal Growth in Cadmium-Induced PE Rats

Given the magnified effect of the complication of FGR on A_{2A} R level in placenta from PE, the foetal growth-related indicators were evaluated as well. Cadmium injection during pregnancy reduced the foetal weight and length and increased the FGR% significantly compared to those in the NP rats (Figure 3A-C, $p < 0.01$). In the SCH+PE rats, the foetal weight and length (Figure 3A and B, $p < 0.05$ and $p < 0.01$, respectively), but not the FGR% (Figure 3C, $p > 0.05$), were improved compared to NP rats.

The Effect of SCH58261 on Placental Development in Cadmium-Induced PE Rats

In view of placental origin of PE, placenta was further assessed. The data suggested that gestational cadmium exposure diminished the placental diameter and placental efficiency (Figure 4A-B, both $p < 0.01$), both of which would be improved by SCH58261 (Figure 4A-B, $p < 0.01$ and $p < 0.05$, respectively). However, there was no difference in placental weight among groups (Figure 4C,

$p > 0.05$). The morphologic changes of the placentae were depicted in Figure 4D. The thickening of the spiral wall was observed in PE but not in NP rats. SCH58261 treatment attenuated the abnormal thickening of the artery wall induced by cadmium but had no significant effect in NP rats. From the results of TUNEL staining (Figure 4E and F), the apoptosis was increased in the PE rats compared to the NP rats (Figure 4D and E, $p < 0.01$), and these effects were reversed in the SCH+PE rats (Figure 4D and E, $p < 0.01$).

The Placental Expression of HIF-1 α and sirt1 in PE Women

After the confirmation of the detrimental role of A_{2A} R in cadmium-induced PE rats, the potential molecular signalling pathway was further explored. As seen from the results of IHC staining (Figure 5A-C), sirt1 (Figure 5A and B, $p < 0.01$) decreased and HIF-1 α (Figure 5A and C, $p < 0.01$) increased in placenta of the PE women by comparison to NP women. These data were showed by the result of WB (Figure 5D-F, both $p < 0.05$).

The Effect of SCH58261 on the Placental Expression of HIF-1 α and sirt1 in Cadmium-Induced PE Rats

As seen from the result of IHC staining and WB (Figure 6A-E), low expression of sirt1 (IHC staining: Figure 6A and C, $p < 0.01$; WB: Figure 6B and E, $p < 0.05$) and high expression of HIF-1 α (IHC staining: Figure 6A and D, $p < 0.01$; WB: Figure 6B and F, $p < 0.05$) were significant in PE rats compared with those in NP rats. After combined with SCH58261 treatment, sirt1 increased (IHC staining: Figure 6A and C, $p < 0.01$; WB: Figure 6B and E, $p < 0.05$) and HIF-1 α (IHC staining: Figure 6A and D, $p < 0.01$; WB: Figure 6B and F, $p < 0.05$) decreased in placenta obtained from SCH+PE rats compared to those in PE rats. The results of RT-qPCR showed that cadmium exposure during pregnancy would inhibit transcriptional expression of sirt1 and increased HIF-1 α although the difference was not significant (Figure 6G-H, both $p > 0.05$). After SCH58261 treatment, the sirt1 mRNA increased (Figure 6G, $p < 0.05$) and HIF-1 α mRNA decreased (Figure 6H, $p > 0.05$).

Discussion

Evidence has closely linked gestational cadmium exposure to placental dysfunctional diseases such as PE and FGR. The published findings also

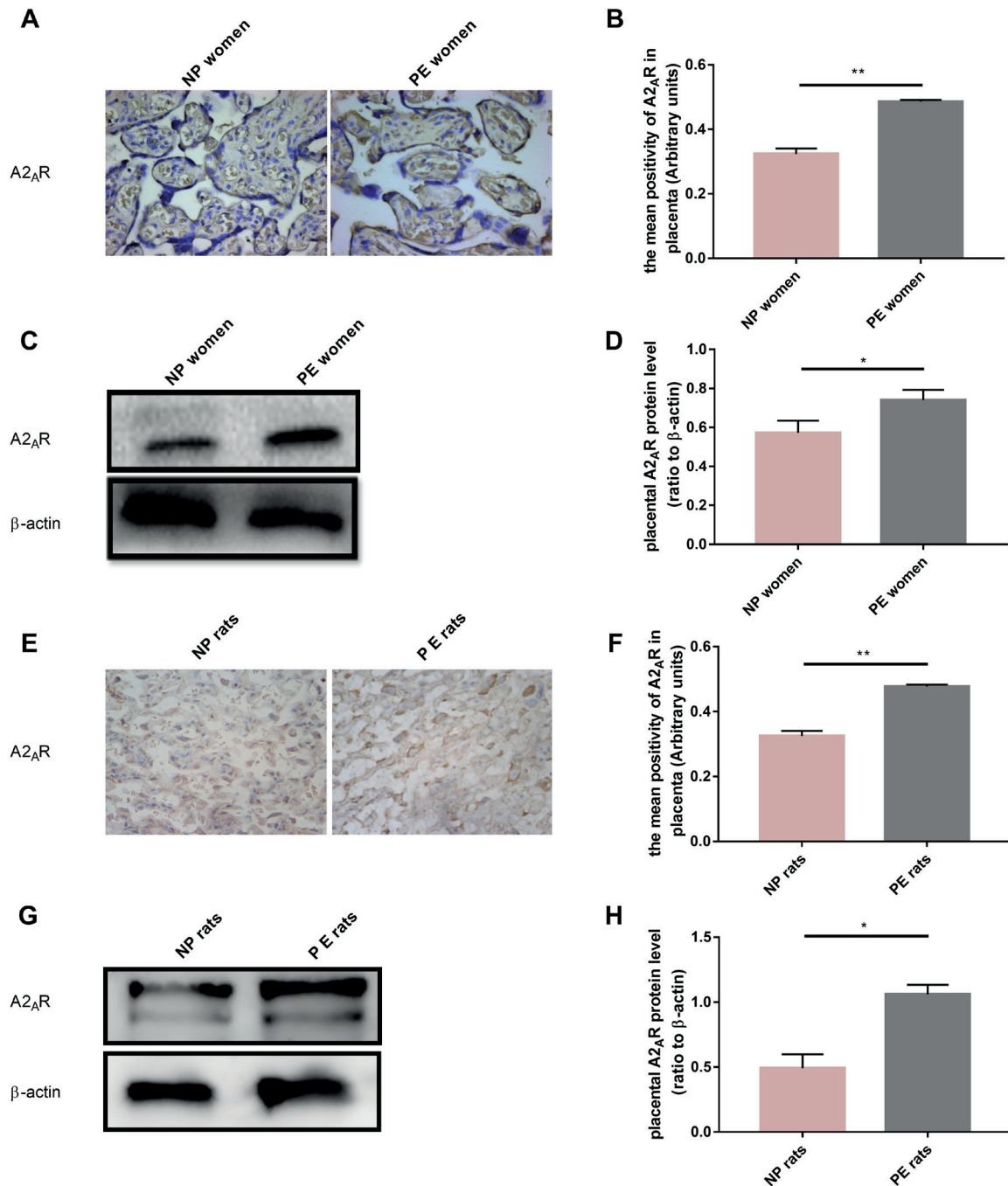


Figure 1. The placental A_{2A}R expression in preeclamptic patients and cadmium-induced preeclamptic rats. **A**, Representative immunohistochemistry (IHC) image showing positive expression of A_{2A}R in placenta of normal pregnancy and PE patients; magnification: 400 \times . **B**, The average IOD of A_{2A}R in placenta in NP and PE women, each N=5. **C-D**, Western blot analysis of the placental expression of A_{2A}R in NP and PE women. **E**, Representative immunohistochemistry (IHC) image showing positive expression of A_{2A}R in placenta of NP and PE rats; magnification: 400 \times . **F**, The average IOD of A_{2A}R in placenta of NP and PE rats, each N=5. **G-H**, Western blot analysis of the placental expression of A_{2A}R in NP and PE rats. Differences between the two groups were assessed by Student's *t*-test (B, D, F, H) (mean \pm SD, **p* < 0.05, ***p* < 0.01).

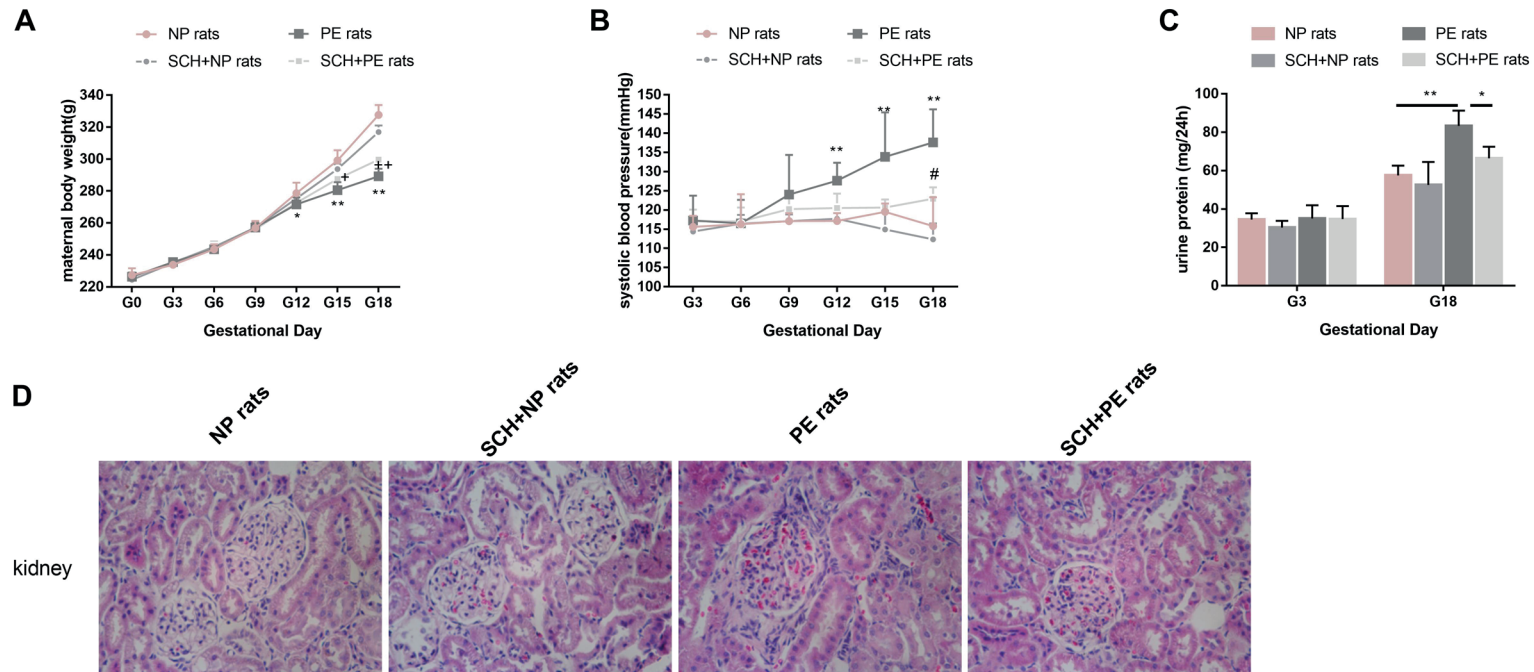


Figure 2. The effect of SCH58261 on the maternal outcome in cadmium-induced PE rats. **A**, Maternal body weight, each N=5 ($*p < 0.05$, $**p < 0.01$ PE rats vs. the NP rats, $+p < 0.05$, $**p < 0.01$ SCH+PE rats vs. PE rats). **B**, Maternal systolic blood pressure, each N=5 ($**p < 0.01$, PE rats vs. NP rats; $#p < 0.05$, $###p < 0.01$ the SCH+PE rats vs. the PE rats). **C**, Twenty-four-hour urine protein level, each N=5. **D**, Representative image showing morphological changes of the kidney; magnification: 400 \times . Differences among groups were assessed by One-way ANOVA (A-C), the results are shown as the mean \pm SD.

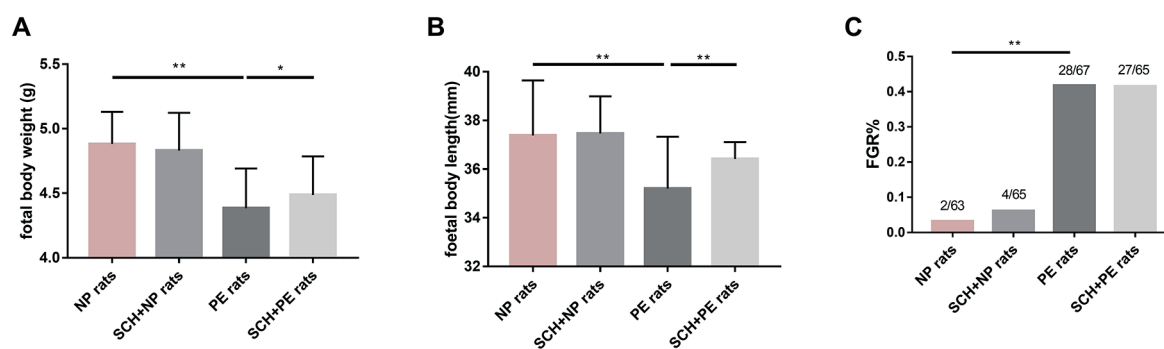


Figure 3. The effect of SCH58261 on foetal growth in cadmium-induced PE rats. **A**, foetal body weight, **B**, foetal body length, **C**, FGR% (the sample sizes showed in Figure 3. **A-C**: NP rats, n=63; SCH+NP rats, n=65; PE rats, n=67; SCH+PE rats, n=65; * p < 0.05, ** p < 0.01). Differences among groups were assessed by One-way ANOVA (**A** and **B**), frequency data were performed by the chi-square test (**C**). The results are shown as the mean \pm SD.

emphasized the importance of A_{2A} R in placental dysfunction, but the connection of cadmium with A_{2A} R in the genesis of PE was still unknown. The present study tried to address the role of placental A_{2A} R and the related potential molecular mechanism in cadmium-induced preeclamptic rats. The data showed that placental A_{2A} R played a part in cadmium-induced preeclamptic rats, and the health benefits of A_{2A} R inhibiting PE development may be attributed to the increased sirtuin-1 (sirt1) and the decreased HIF-1 α in placenta.

It is well known that cadmium exposure during pregnancy contributes to the development of PE. However, the exact mechanism remains unclear. The available data have shown that cadmium exposure would cause a significant increase in ectonucleotidases which not only would cause an increase of adenosine, but also would affect the action and affinity of adenosine receptors^{8,9,29}. The abnormal activation of adenosine signal has been identified to account for the pathogenesis of PE, for which ADA deficient mouse could spontaneously develop into PE, FGR, and even into still-birth during pregnancy^{13,18,30}. During pregnancy, all adenosine receptor subtypes would increase in the placenta, but A_{2A} R is the only subtype which increases significantly under hypoxia *in vitro*¹⁷. This receptor has also been hypothesized as a potential pathogenic factor in the course of PE^{11,16}. However, no prior research has been done on the relationship between cadmium exposure and A_{2A} R in the course of PE. This study firstly found that the expression of A_{2A} R remarkably increased in placental biopsies obtained from the pregnancy complicated by PE, as well as from cadmium-induced preeclamptic rats. In contrast to our results,

Escudero et al³¹ showed a decrease in A_{2A} R expression in placenta. However, that study focused on the A_{2A} R expression in the placental endothelium and not whole placental tissue, which might explain this difference.

There was still no well-recognized model for PE. The model used in this study was established entirely based on our previous study²⁰. This model has been well proved to be able to imitate PE-like manifestations including hypertension and proteinuria, and lead to FGR. The model built in the present study replicated the typical changes of PE very well that the SBP, proteinuria increased, the morphology of the placenta and kidney changed significantly in PE group, which is consistent with the previous study^{32,33}. Given that placenta is the main target of cadmium, the placental growth was measured. Literatures suggested that the placental growth could be reflected by placental diameter, weight, efficiency, and apoptosis³⁴⁻³⁶. In our study, the placental diameter was reduced in the PE rats, which was consistent with the reports that the placental diameter in preeclamptic patients was smaller than normal pregnancy women^{34,35}. The placental efficiency, the main indicator of the function and transportability of this organ, is closely related to the foetal growth, and its reduction echoed the increase of FGR% in PE rats²⁶. Visually, the placental apoptosis also remarkably increased in PE group, which was consistent with the damage effect of cadmium on trophoblast³⁷. Although there was no significant difference in placental weight between the PE and NP groups, it kept pace with our previous study²⁰.

Placenta origins of PE have been well-established in academia^{38,39}. A_{2A} R, a quick and respon-

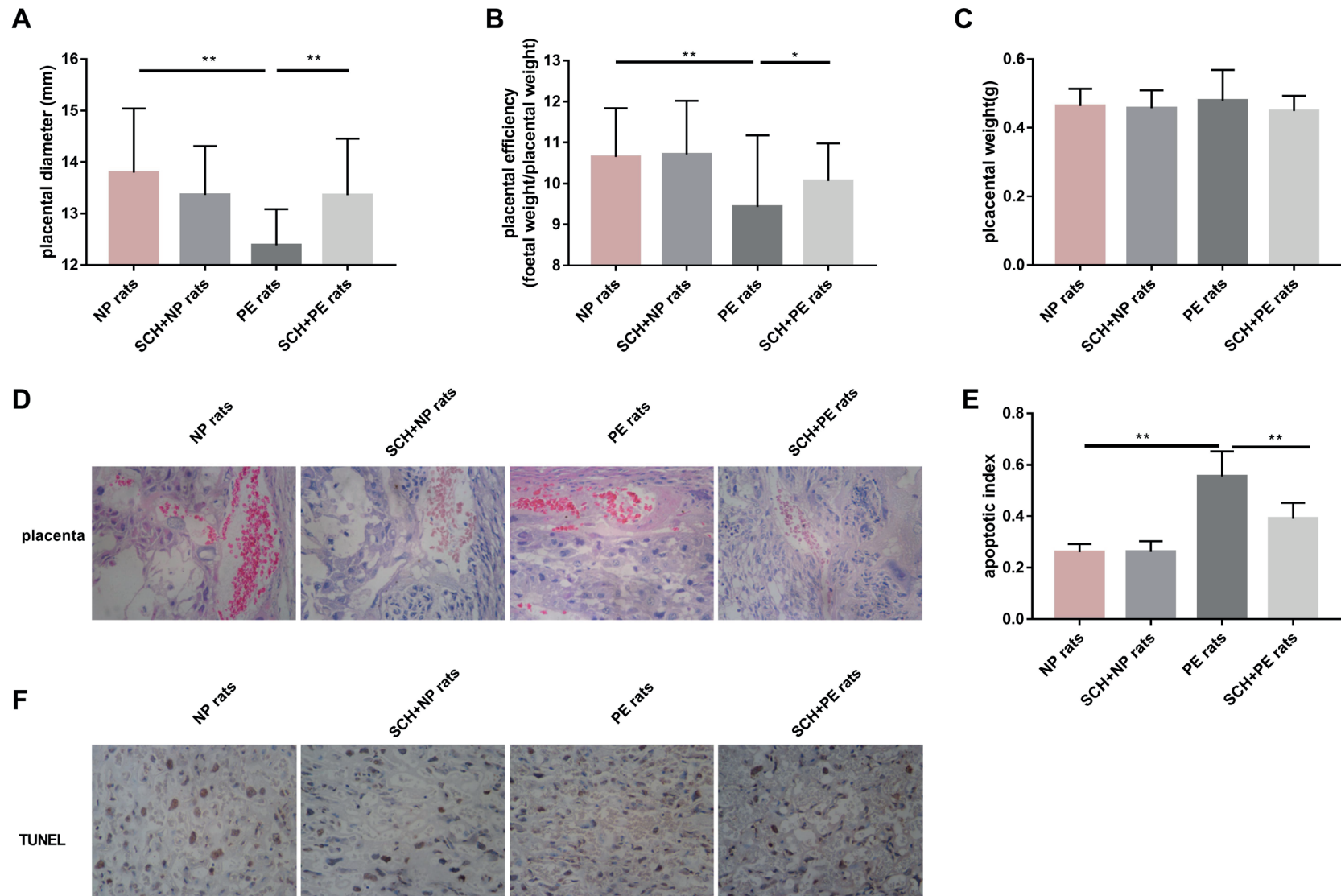


Figure 4. The effect of SCH58261 on placental development in cadmium-induced PE rats. **A**, placental diameter. **B**, placental efficiency. **C**, placental weight (the sample sizes showed in NP rats, n=63; SCH+NP rats, n=65; PE rats, n=67; SCH+PE rats, n=65; * $p < 0.05$, ** $p < 0.01$). **D**, Representative placental image of TUNEL staining; magnification: 400 \times . **E**, The apoptotic index of placenta; each N=5. Differences among groups were assessed by One-way ANOVA (A-C), frequency data were performed by the chi-square test (E) (mean \pm SD, * $p < 0.05$, ** $p < 0.01$).

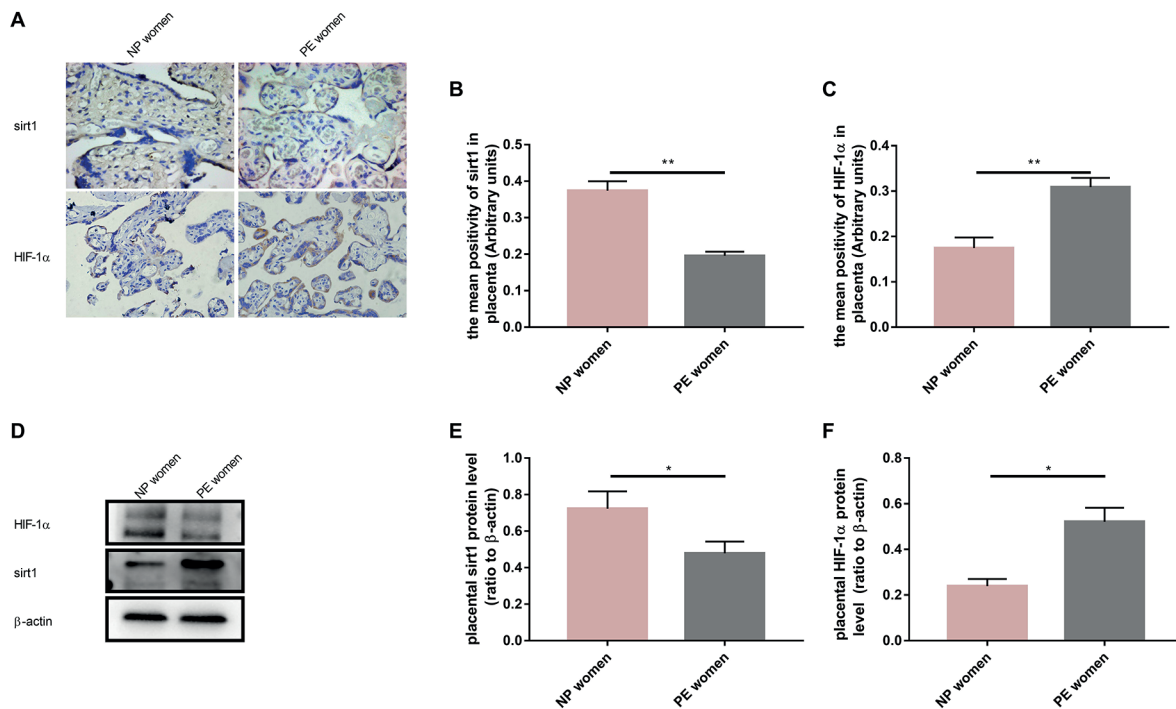


Figure 5. The placental expression of HIF-1 α and sirt1 in PE women. **A**, Representative IHC image showing the expression of sirt1 and HIF-1 α in placenta in NP and PE women (Magnification $\times 100$). **B-C**, The average IOD of sirt1 and HIF-1 α in placenta of NP and PE women; each N=5. **C**. **D-F**, Western blot analysis of the placental expression of sirt1 and HIF-1 α in NP and PE women. Differences among groups were assessed by One-way ANOVA (C-H) (mean \pm SD, * $p < 0.05$, ** $p < 0.01$).

sive sensor of the tissue damage, catches researchers' attention in the study of PE. In the present research, after the identification of the abnormal increase of the $A_{2A}R$ in cadmium-induced PE, its specific antagonist, SCH58261 was used to check the role of $A_{2A}R$ in cadmium-induced preeclamptic model. The data firstly reported the beneficial effect of $A_{2A}R$ on maternal outcomes, foetal and placental growth. However, there was no statistically significant difference in FGR% between PE and SCH+PE rats. Momoi et al⁴⁰ showed that MSX-3, an $A_{2A}R$ antagonist, could induce FGR, whose dosage of the antagonist used in the study was much larger than ours, which not only may account for the difference⁴¹, but also may imply the bidirectional role of $A_{2A}R$ in placental dysfunctional disease by dose-dependent method. The conclusion needs further research.

Most investigations on the negative role of $A_{2A}R$ in the course of PE have linked the abnormal elevation of $A_{2A}R$ to HIF-1 α . HIF-1 α activation is considered as a fundamental factor in the development of PE, and the promotion of cadmium on the expression of HIF-1 α in human placental trophoblast has been perfectly demon-

strated in the past studies²². It was found that $A_{2A}R$ is positive correlated with HIF-1 α in human placenta during pregnancy^{10,16}, and the coordination between $A_{2A}R$ and HIF-1 α participates in sustaining tissue damage^{19,42,43}. Consistent with it, the placental expression of HIF-1 α protein was increased along with $A_{2A}R$ in PE rats in our data. On the contrary, the placental HIF-1 α was inhibited remarkably after SCH58261 treatment. In our earlier research, it had been demonstrated that gestational cadmium exposure accounted for the abnormal immune adaptation in the development of PE²⁰. Not only that, the role of $A_{2A}R$ -HIF-1 α axis in immune suppression has attracted plenty of attention, whose activation is likely to shift immune function to generate chronic inflammation in the development of PE^{19,44-46}. The findings conduct us to link the abnormal $A_{2A}R$ elevation to immunological maladaptation. In addition, there was no significant difference of the placental expression of HIF-1 α in transcriptional expression, which may attribute to the limited sample size.

Sirt1, an NAD⁺-dependent deacetylase, is a protecting molecule to prevent tissue from damage such as oxidative stress-mediated injury⁴⁷⁻⁴⁹.

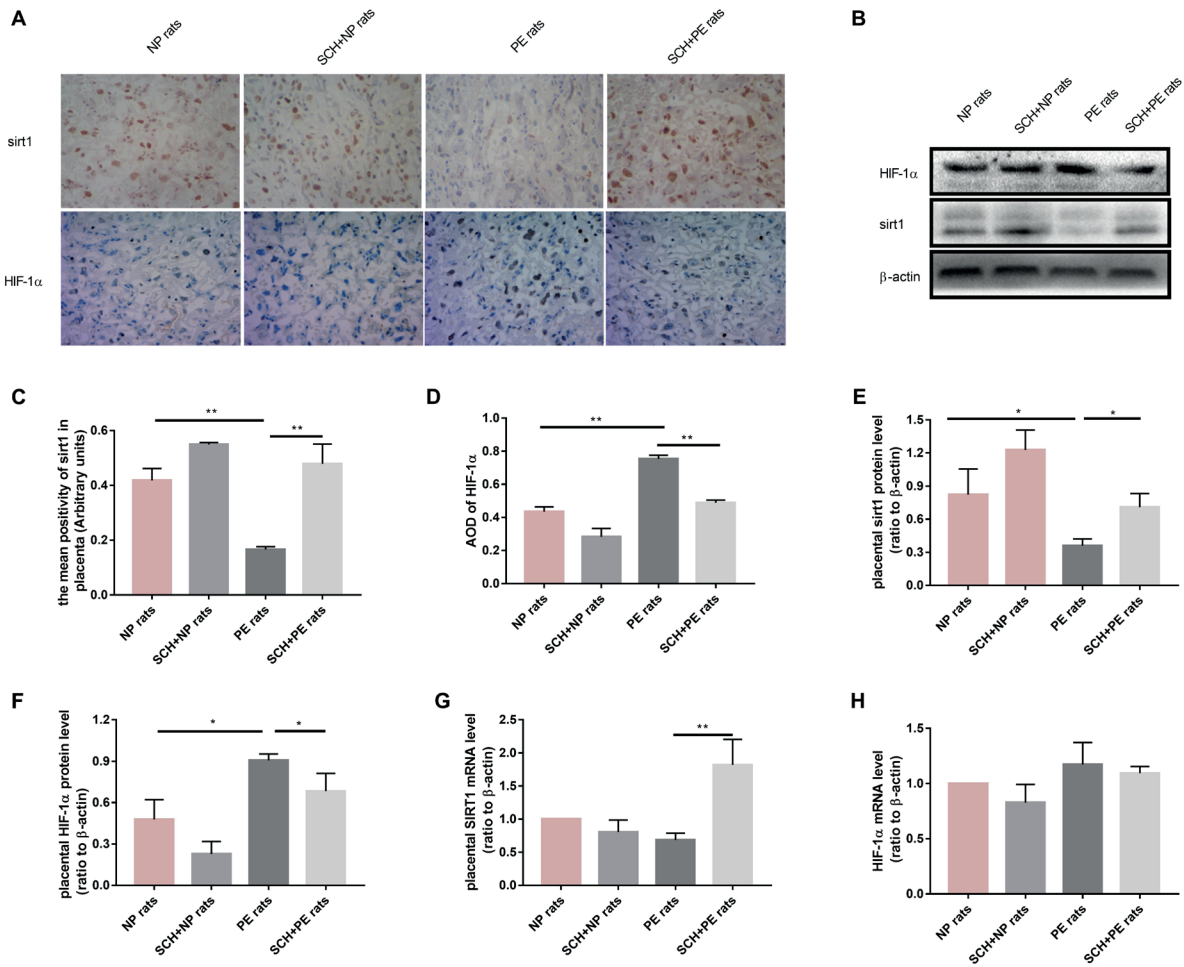


Figure 6. The effect of SCH58261 on the placental expression of HIF-1 α and sirt1 in cadmium-induced PE rats. **A**, Representative IHC image showing the placental expression of sirt1 and HIF-1 α among groups in rats. **B**, **E**, and **F**, Western blot analysis of the placental expression of sirt1 and HIF-1 α among groups in rats. **C**–**D**, The average IOD of sirt1 and HIF-1 α in placenta among groups in rats. **G**–**H**, RT-qPCR analysis of sirt1 and HIF-1 α among groups in rats. Differences among groups were assessed by One-way ANOVA (C–H) (mean \pm SD, * p < 0.05, ** p < 0.01).

Its reduction in placenta could cause FGR, placental impairment and accelerate the progression of PE^{50,51}. Cadmium exposure is considered as a damaging factor for sirt1 expression in various organs, such as kidney, liver, and brain⁵²⁻⁵⁴. However, no earlier study has inspected the effect of cadmium on the expression of sirt1 in the placenta. Our data presented that both transcriptional expression and protein level of sirt1 were inhibited in cadmium-induced preeclamptic rats. The negative directed regulation of sirt1 on HIF-1 α in multiple pathophysiological processes, such as oxidative stress, immunity, proliferation, and cell growth, has been recognized in numerous papers. All the aforementioned processes were found in the genesis of PE in past studies. Thus, no specific

processes have been detected including oxidative stress. Similar to A_{2A}R, sirt1 is a pivotal factor participating in the regulation of immune maladaptation, but its downregulation would polarize T cell into pro-inflammatory phenotype via HIF-1 α pathway⁵⁵. The finding implied an opposite role of A_{2A}R and sirt1 in the regulation of the equilibrium point of HIF-1 α . However, will A_{2A}R inhibition has a direct action on sirt1 expression? In fact, the A_{2A}R suppression has been hypothesized to participate in improving mitochondrial NAD⁺ activation⁵⁶, which may directly increase the production of sirt1. The study firstly found that the protein and mRNA of sirt1 in placenta increased with SCH58261 treatment in cadmium-related preeclamptic rats. All the results suggested there

may be a complex regulatory system among A_{2A}R, sirt1, and HIF-1 α in the course of PE. Further studies are needed for the novel hypothesis.

The role of the mitochondria deserves attention in the pathological physiology of PE for the following reasons. First, prenatal cadmium-induced immune maladaptation has been verified as a pivotal event in human and pregnant rats who suffered from PE²⁰. Second, the A_{2A}R and sirt1 are the primary factors in mediating metabolic stress and immune maladaptation, whose action relies on the intact mitochondrial function. What also deserves attention is the different distributed localization of sirt1 in human and rats' trophoblast. It was noticed that sirt1 distributed in the nuclei of trophoblasts in rats while in the cytoplasm of trophoblasts in humans, and the results are in accordance with the previous data found in the corresponding species^{51,57}. It was reported that the different localization of sirt1 linked with different function, but no shuttling of sirt1 between the nucleus and cytoplasm in the trophoblast has been reported, which may also need further study or a more sensitive detection method.

Conclusions

To the best of our knowledge, for the first time, it has been demonstrated that placental A_{2A}R mediated the pathological progression of PE induced by cadmium, in which sirt1/HIF-1 α may play an essential role. This finding is expected to provide a target for early monitoring and treatment for PE patients. However, further research is needed to elucidate the novel hypothesis in the development of PE.

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Conflict of Interest

The authors certify that there is no competing financial conflict of interests.

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