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Is Atrial Natriuretic Peptide (ANP) and Natriuretic Peptide Receptor-A (NPR-A) Expression in Human Placenta and Decidua Normal?

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Atrial natriuretic peptide (ANP) is a cardiac hormone that regulates blood pressure and the salt-water balance in the blood. It acts through natriuretic peptide receptors (NPR), and the major biologically active ANP receptor is natriuretic peptide receptor-A (NPR-A). Aberrant forms of ANP and its receptors have been reported in patients with preeclampsia. However, whether aberrant forms of ANP or NPR-A are present in preeclamptic placenta, and what their role is in preeclampsia pathogenesis, has not yet been elucidated clearly. The aim of this study was to assess the expression of ANP and NPR-A in the placenta and decidua and its role in preeclampsia development.

Material/Methods: The expression of ANP and NPR-A in the first-trimester villous and decidua, full-term placenta, and preeclamptic placenta was determined using immunohistochemistry and Western blot analysis. The HTR8/SVneo cell line was used to investigate the role of NPR-A in proliferation, apoptosis, and invasion using Cell Counting Kit-8 analysis, flow cytometry analysis, and a Transwell invasion assay, respectively.

Results: ANP and NPR-A were localized in the syncytiotrophoblasts, cytotrophoblasts, and trophoblast columns of human first-trimester villous trophoblast cells of decidua, and in the glandular epithelium and extravillous trophoblast cells of decidua. ANP-positive and NPR-A-positive cells in the decidual stroma were clustered around and infiltrated into the vascular wall of the spiral artery undergoing remodeling. NPR-A expression was significantly reduced in preeclamptic placentas, and NPR-A knockdown significantly impaired the invasion ability of HTR8/SVneo cells, although it had no effect on cell proliferation and apoptosis.

Conclusions: ANP and NPR-A are involved in human placental development. Decreased levels of NPR-A may contribute to the development of preeclampsia.

MeSH Keywords: Decidua • Placenta • Pre-Eclampsia • Trophoblasts

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Background

Preeclampsia (PE) is one of the serious complications of pregnancy, and it is characterized by a first occurrence of hypertension after 20 weeks of gestation [1]. It is a substantial contributor to maternal and perinatal morbidity and mortality worldwide, especially in developing countries [2,3]. However, there is still a lack of measures of prevention and effective treatment, due to the fact that the underlying pathogenesis is complicated and not yet well clarified. Accumulating evidence indicates that PE is a placenta-borne disease. Abnormal placental development resulting from insufficient trophoblast invasion in the first trimester of pregnancy plays a central role in the pathogenesis of PE [4].

Atrial natriuretic peptide (ANP) is a member of the atrial natriuretic peptide family, which is mainly secreted by the cardiac atria and plays an important role in regulating blood pressure and maintaining the electrolyte balance and extracellular fluid volume [5]. ANPs exert their biological activities through the natriuretic peptide receptors (NPRs) [6]. Natriuretic peptide receptor-A (NPR-A) is the major biologically active receptor for ANP. It contains an intracellular guanylyl cyclase domain, which produces cGMP as a second messenger in response to ANP binding. Next, cGMP activates cGMP-dependent protein kinase (PKG), which in turn can activate different downstream effectors involved in cell growth, apoptosis, proliferation, and inflammation [7].

Prior studies have shown that ANP and its receptors are important in pregnancy maintenance, and aberrations of ANP and its receptors exist in patients with PE. It has been reported that ANP levels increase significantly in the maternal peripheral blood after the onset of pregnancy, and further increase in patients with preeclampsia [8]. Recently, Yang et al. reported that NPR-A expression was reduced in the maternal vessel endothelium in patients with preeclampsia [9]. Studies of the placenta have found that ANP receptors were reduced in human placenta from growth-restricted fetuses, and the affinity of ANP for its receptors was significantly decreased in preeclamptic placenta [10,11]. However, these studies of the placenta have not yet elucidated what type of natriuretic peptide receptor is abnormal in preeclamptic placenta. Most studies on the role of ANP and its receptors in pregnancy maintenance and PE pathogenesis have emphasized their impact on the regulation of maternal and placental hemodynamics. Recently, mounting evidence has highlighted an important role of ANP and NPR-A in mouse placental development. It has been reported that ANP knockout mice suffered from hypertension after pregnancy onset, and that their placenta exhibited insufficient trophoblast invasion and abnormal uterine spiral arterial remodeling [12]. NPR-A knockout mice exhibited abnormal embryonic vascular development and ischemia at the fetal surface of the placenta [13]. Moderate trophoblast invasion is known to play an important role in placental development. Studies in the field of oncology have found that ANP and NPR-A are important in various tumor cell functions such as proliferation, invasion, migration, and apoptosis [14,15]. It has been established that many factors and signals involved in tumor invasion play roles in trophoblast function. Thus, we believe that ANP and NPR-A are also involved in regulating trophoblast function. We speculated that: 1) ANP and NPR-A may also play a role in human placental development, and 2) aberrant forms of ANP and/or NPR-A exist in preeclamptic placentas, and they may participate in the development of PE by affecting trophoblast function.

To test our hypothesis, we assessed: 1) the localization of ANP and NPR-A expression in first-trimester and full-term placenta and early-pregnancy decidual tissue by immunohistochemistry, so as to analyze its relationship to placental development; and 2) whether aberrations in ANP and/or NPR-A expression exist in the placenta of PE patients by use of Western blot analysis, and what their effects are on trophoblasts by use of HTR8/SVneo cell lines.

Material and Methods

Tissue collection and processing

Full-term placenta samples were obtained from the hospital from women (n=10) with severe preeclampsia and from women (n=10) with normal pregnancies. The criteria for the diagnosis of severe preeclampsia were based strictly on the guidelines of the American College of Obstetrics and Gynecology. Patients with chronic hypertension, diabetes mellitus, renal disease, polycystic ovarian syndrome, fetal malformations, and multiple pregnancies (≥3 fetuses), or alcohol and drug addiction were excluded from this study. Each sample was divided into 2 blocks per patient, washed in PBS, and then 1 block was stored at -80°C for the Western blot analysis, and the other block was fixed in 10% neutral buffered formalin and embedded in paraffin wax for immunohistochemistry.

The first-trimester decidua and villous samples were collected from women showing a normal pregnancy (n=10) undergoing surgical elective terminations at 8 to 12 weeks gestation in the hospital for psychosocial reasons. All samples were washed in PBS, fixed in 10% neutral buffered formalin, and then embedded in paraffin wax for immunohistochemistry.

The study was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University, Guangzhou, China (23rd Nov 2013).

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Immunohistochemistry (IHC)

The sections were incubated with anti-human-ANP (1: 150; Abcam, San Francisco, CA), -NPR-A (1: 200; Abcam, San Francisco, CA), -SMA (1: 150; Boster Biological Technology, Wuhan, China), -CK-7 (1: 150; Boster Biological Technology, Wuhan, China), and -HLA-G (1: 150; Bioss Biological Technology, Beijing, China) primary antibodies overnight at 4°C. The next day, they were incubated with secondary antibody (goat anti-rabbit, 1: 1,000; Zhongshan Biotechnology, Beijing, China) for 30 min. All sections were developed using peroxidase-conjugated streptavidin and diaminobenzidine agent and then counterstained with hematoxylin. Finally, the slides were mounted with a cover glass and evaluated using an Olympus BX51 light microscope.

Western blotting

Briefly, 10 mg placenta were ground in liquid nitrogen and transferred into EP tubes. Then, radioimmunoprecipitation assay lysate buffer was added to the tissues, which were homogenized on ice using an Ultrasonic Cell Disruptor and then centrifuged at 16 000 rpm for 30 min at 4°C. The protein concentrations were measured using the Pierce BCA Protein Assay Kit (KeyGen Biotech, Nanjing, China). Next, the membranes were incubated with diluted primary antibody (ANP, 1: 150; Abcam, San Francisco, CA; NPR-A, 1: 200; Abcam, San Francisco, CA).

Subsequently, the membranes were incubated with secondary antibody (goat anti-rabbit, 1: 1,000; Zhongshan Biotechnology, Beijing, China) and then developed using an ECL Western Blotting Detection System. Band intensities were calculated using the AlphaEaseFC FluorChem 8900 software (Alpha Innotech Corporation, San Leandro, CA).

Cell line culture

A first-trimester extravillous trophoblast cell line (HTR-8/SVneo cells), received from Dr. Charles H. Graham (Queen's University, Ontario, Canada), was used for subsequent experiments. The cells were cultured in RPMI-1640 (Gibco, MA) supplemented with 10% fetal bovine serum (FBS; Gibco, Australia), 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco, Australia) at 37°C and 5% carbon dioxide (CO_2).

RNA interference

RNA interference was carried out in the HTR-8/SVneo cells to examine the effect of NPR-A on trophoblast cell function, using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) in 6-well plates according to the manufacturer's protocol. Briefly, the cells were seeded, incubated overnight, and transfected with NPR-A-siRNA (GenePharma Technology, Shanghai, China) and NC-siRNA (a universal NC; GenePharma) at a final concentration of 40 nM for 24 h. At 48 h after transfection, knockdown efficiency was confirmed by Western blotting. The target sequences of the NPR-A-siRNAs were as follows: sense, 5'-GACACACCGUUUAUGCCAATT-3'; anti-sense, 5'-UUGGCAUAAACGGUGUGUCTT-3'.

Invasion assays

Invasion assays were performed in 24-well plates to assess the effect of NPR-A on the HTR8/SVneo cell line. HTR8/SVneo cells were cultured in 6-well plates and transfected with NC-siRNA or NPR-A-siRNA for 48 h. Next, a total of 1×10^5 HTR8/SVneo cells in 200 µl of RPMI-1640 containing 2% FBS were plated in the upper compartments of Transwell inserts coated with Matrigel (BD Biosciences, San Jose, California), while the lower insert compartments were filled with 600 µl of RPMI-1640 containing 10% FBS. Cells were cultured for 24 h at 37°C in 5% CO_2.

After incubation, cells in the upper inserts were removed on cotton swabs, fixed in 4% buffered neutral formalin, and the invaded cells were counted under a microscope (Olympus IX51) at a magnification of 200× in 5 random fields.

Flow cytometric analysis of apoptosis

An Annexin V-FITC and PI Apoptosis Detection Kit (Sungene Biotech, Tianjin, China) was used to evaluate the apoptosis rate in HTR8/SVneo cells. In brief, 48 and 72 h after transfection with NPR-A-siRNA and NC-siRNA, cells were harvested and stained with Annexin V/PI according to the manufacturer's instructions. The apoptosis rate was detected by using flow cytometry according to the manufacturer's guidelines (BD Biosciences).

Cell proliferation test

Cell growth was measured by a Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) in accordance with the manufacturer's instructions. Briefly, HTR8/SVneo cells pre-treated with NPR-A-siRNA and NC-siRNA were seeded at the same density on a 96-well plate and cultured for 48 and 72 h. Next, the cells in the 96-well plate were incubated with 10 µl CCK-8 in 100 µl of PRMI-1640 medium in the dark for 1 h, and the OD value at 450 nm was detected by a microplate reader.

Statistical analysis

The statistical analysis was performed using the SPSS software package, version 13.0.

Numeric data are expressed as the mean ± standard deviation. Statistical differences were calculated using the *t* test (for 2 groups) or one-way ANOVA (for more than 2 groups)

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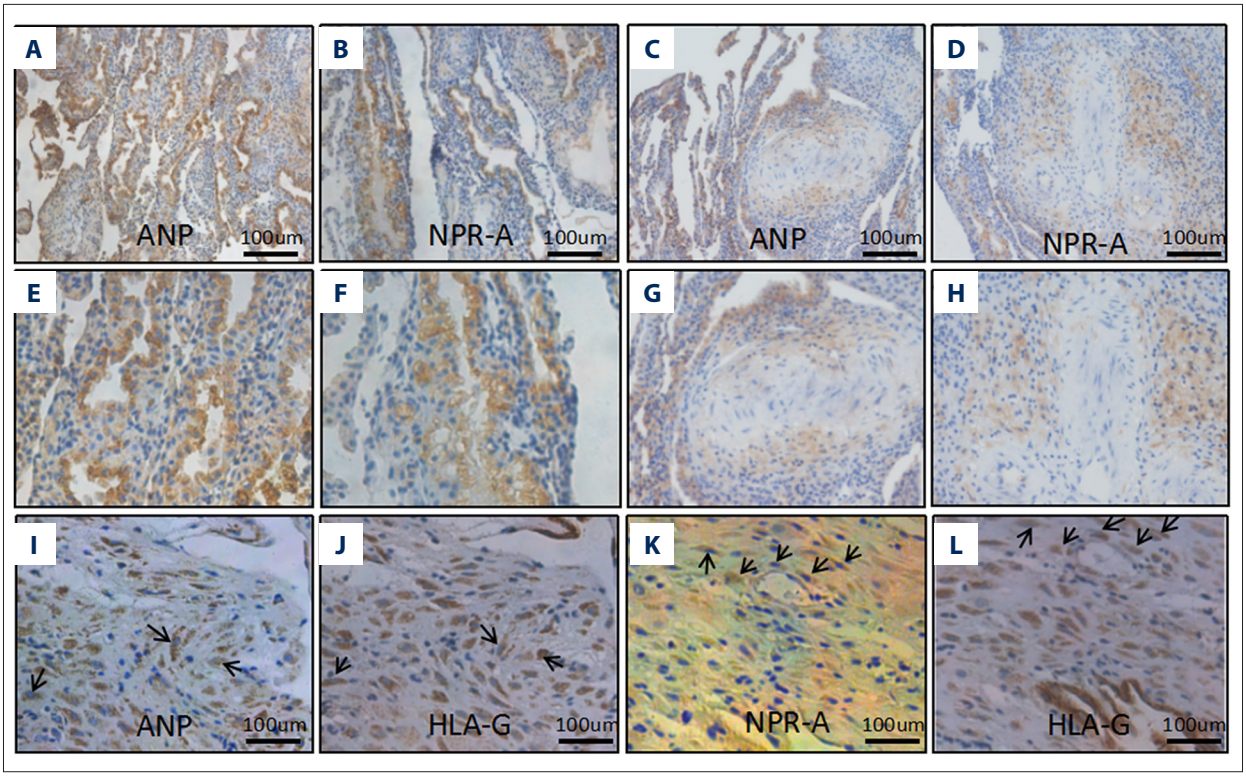

Figure 1. Localization of ANP and NPR-A expression in human first-trimester decidua. A-I: Immunostaining of ANP and NPR-A in first-trimester decidua. ANP (**A**) and NPR-A (**B**) were localized to the glandular epithelium. ANP-positive (**C**) and NPR-A positive (**D**) cells clustered around uterine spiral artery. (**E–H**) High-powered images of **A–D**. (**I–L**) Immunostaining for ANP, NPR-A, and HLA-G in serial sections. ANP (**I**) and NPR-A (**K**) was strongly expressed in some extravillous trophoblasts (EVT). (**J, L**) Immunostaining for HLA-G as marker of EVT in the maternal decidua. ANP – atrial natriuretic peptide; NPR-A – natriuretic peptide receptor A; HLA-G – human leukocyte antigen G.

when the data were normally distributed, or a nonparametric Mann-Whitney U test if the data distribution of any group was skewed. A P value <0.05 was considered statistically significant.

Results

Localization of ANP and NPR-A in basal decidua during early pregnancy

As the basal decidua play an important role in placental development, particularly trophoblast invasion, an immunohistochemistry assay was conducted to examine the localization of ANP and NPR-A in human basal decidua. Immunostaining for HLA-G was used to identify basal decidua containing EVT invasion, and α-SMA staining was used to identify the decidual arteries.

ANP-positive and NPR-A-positive cells were both found to be strongly expressed in the glandular epithelium and abundantly distributed throughout the decidual stroma (Figure 1A–1H), and in particular to be clustered around and infiltrated into the vascular wall of the spiral artery undergoing remodeling (Figure 1C, 1D; Figure 2A–2L). Immunostaining for ANP and NPR-A in serial sections showed that HLA-G-positive trophoblast cells were also ANP- and NPR-A-positive (Figure 1I–1K).

Localization of ANP and NPR-A in the first-trimester villous and full-term and preeclamptic placenta

ANP and NPR-A were found to be expressed in the syncytiotrophoblasts (STB), cytotrophoblasts (CTB), and trophoblast columns (TC) of first-trimester placental villous (Figure 3A–3C). ANP and NPR-A were both expressed in the syncytiotrophoblasts in normal full-term and preeclamptic placentas. The immunostaining of NPR-A in full-term placenta was reduced compared to preeclamptic placenta (Figure 4A).

NPR-A expression was decreased in the placenta of women with severe PE

To further assess the association of ANP and NPR-A with the development of severe preeclampsia, ANP and NPR-A protein expression in the placenta was quantified by Western blotting (Table 1).

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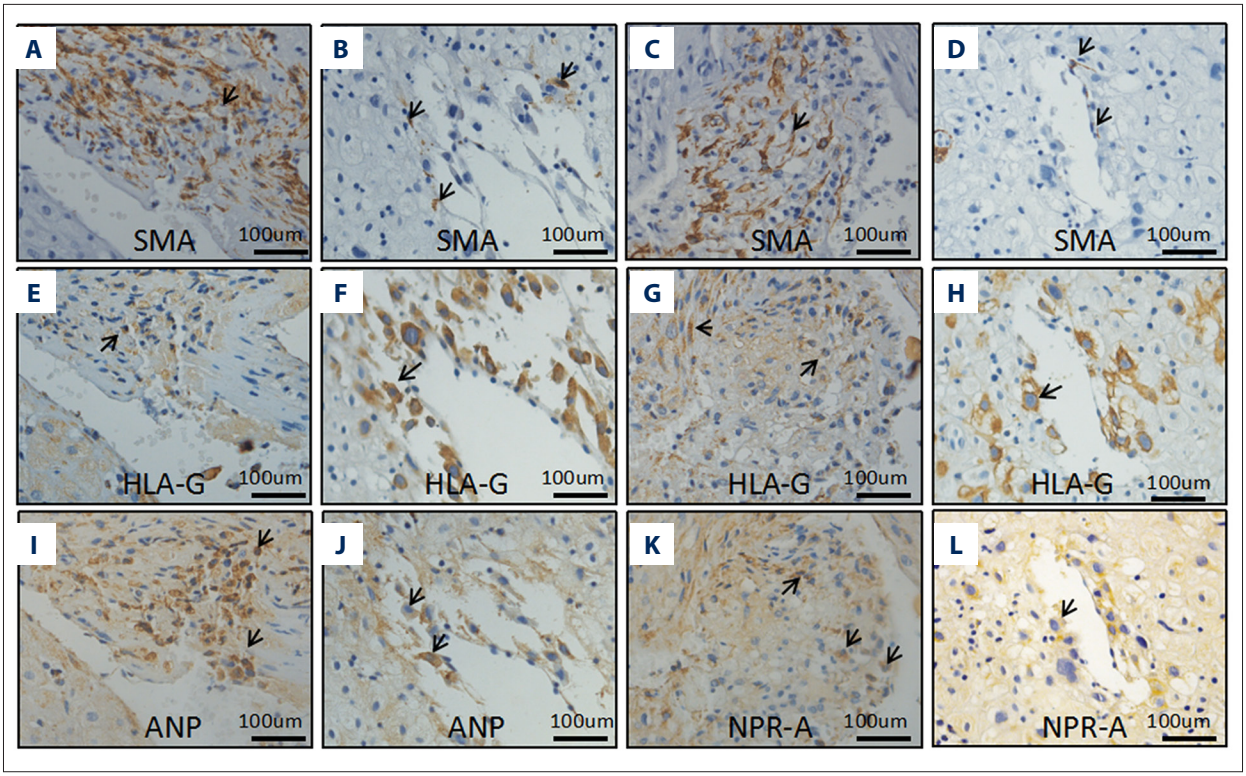

Figure 2. The association of ANP and NPR-A with vascular remodeling. (**A–D**) Remodeling vessels were identified by immunostaining sections with SMA and HLA-G in serial sections. Remodeling vessels displayed dramatically disrupted or disorganized VSMC (**A, C**) or substantial VSMC loss (**B, D**) and EVT infiltration into the vascular wall (**E, G**), or presence of EVT within the vessel lumen (**F, H**). (**I–L**) ANP-positive (**I**) and NPR-A-positive (**K**) cells infiltrated into the vascular wall of spiral artery wall undergoing remodeling. ANP-positive (**I**) and NPR-A-positive (**K**) cells were present within the vessel lumen of spiral artery wall undergoing remodeling. ANP – atrial natriuretic peptide; NPR-A – natriuretic peptide receptor A; HLA-G – human leukocyte antigen G; VSMC – smooth muscle actin.

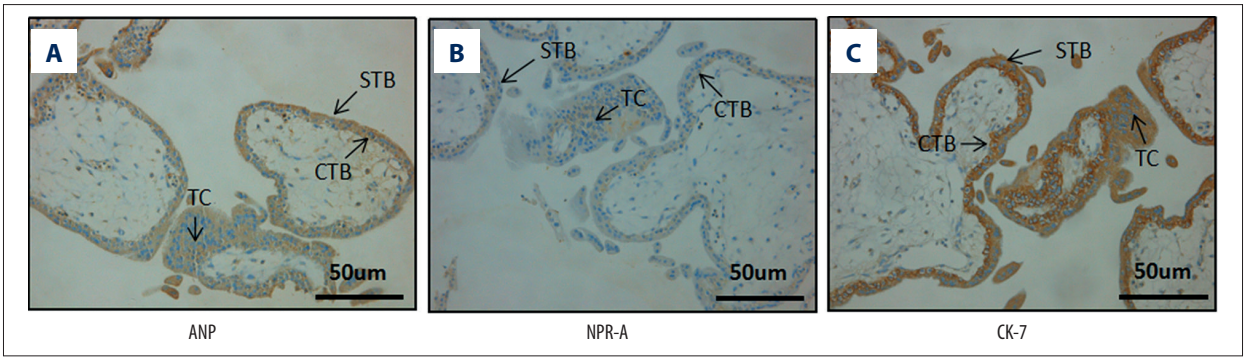

Figure 3. Expression of ANP and NPR-A in first-trimester placenta. ANP (**A**) and NPR-A (**B**) localized to STB, CTB, and TC in human placental villous. (**C**) Immunostaining for CK7 as a marker of STB, CTB, and TC in the first-trimester placental villous. ANP – atrial natriuretic peptide; NPR-A – natriuretic peptide receptor A; STB – syncytiotrophoblasts; CTB – cytotrophoblasts; TC – the trophoblast columns; CK7 – cytokeratin7.

There was no significant difference in maternal age, BMI, and gestational age between the 2 evaluated pregnancy groups. However, the women with PE had significantly higher systolic and diastolic blood pressure and urinary protein quantity at 24 h.

NPR-A expression in preeclamptic placenta was significantly lower than in normal placenta (P=0.000; Figure 4D). However, ANP expression was similar in preeclamptic and normal placenta (P=0.582; Figure 4B, 4C).

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Table 1. Clinical data between patients with severe PE and normal controls.

Characteristics	Severe PE group	Normal control group	P value
Maternal age, years	29.50±6.78	31.12±4.45	0.580
Gestational age, week	36.00±3.42	38.75±1.28	0.052
Maternal BMI	21.39±2.00	22.74±2.21	0.224
SBP, mmHg	160.12±19.72	122.37±10.86	0.000*
DBP, mmHg	108.62±11.42	74.75±9.43	0.000*
24h urine protein, grams	5.87±4.45	0.00±0.00	0.002*
Birth weight, g	2382.50±1036.61	3215.12±631.24	0.073

BMI – body mass index; DBP – diastolic blood pressure; SBP – systolic blood pressure; SD – standard deviation. Data are presented as the mean ±SD. P value <0.05 was considered statistically significant.

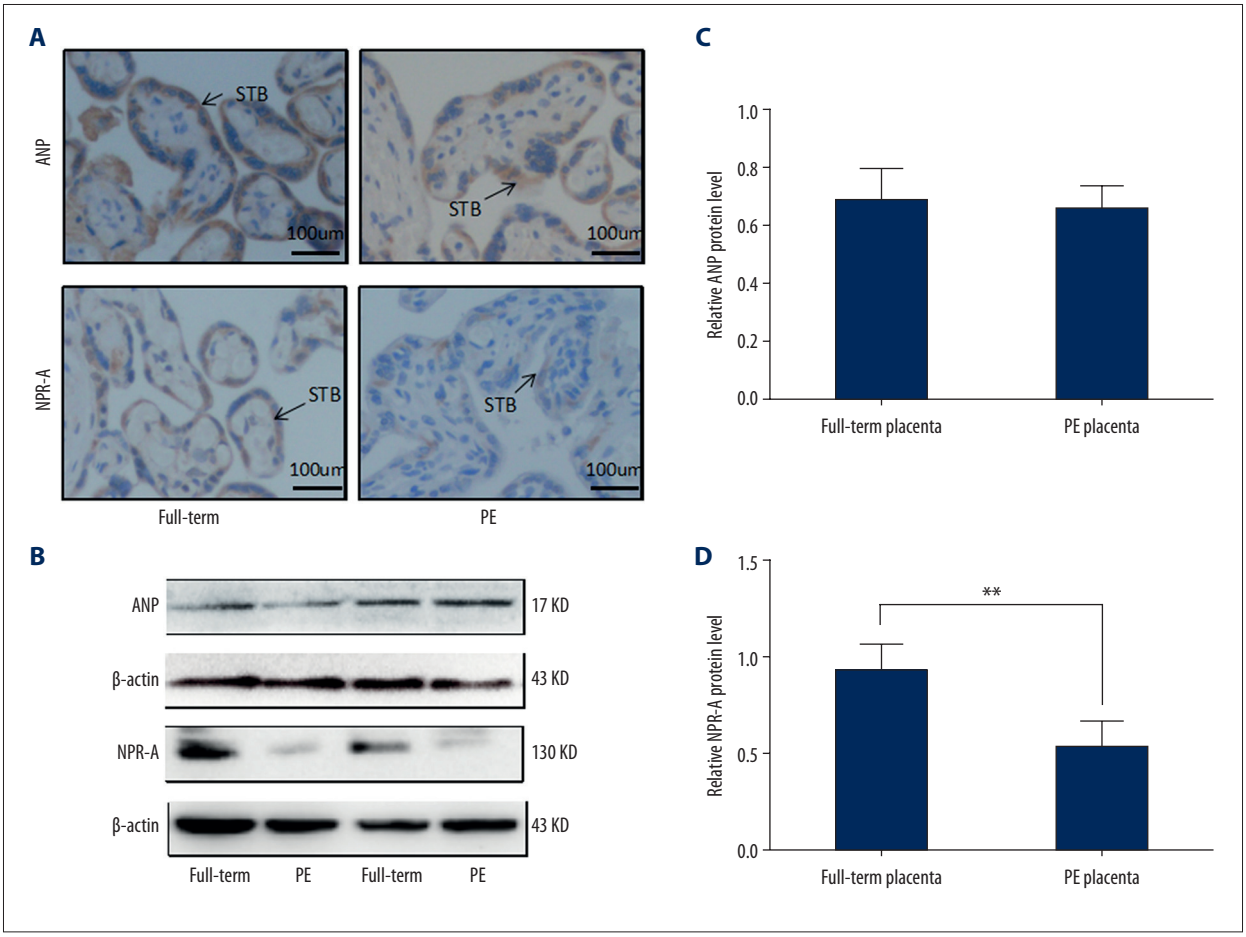

Figure 4. (**A–D**) Expression of ANP and NPR-A in full-term and preeclamptic placenta. (**A**) Immunostaining of ANP and NPR-A in full-term and preeclamptic placenta. ANP and NPR-A were strongly expressed in STB and CTB in full-term placenta. NPR-A levels were decreased in preeclamptic placenta. (**B**) Western blot analysis of ANP and NRP-A protein expression. (**C**) Relative ANP and NPR-A protein expression as assessed by densitometry analysis and normalized to β-actin protein expression; ** P<0.01. The values are given as means (SD). ANP – atrial natriuretic peptide; NPR-A – natriuretic peptide receptor A; STB – syncytiotrophoblasts; CTB – cytotrophoblasts; SD – standard deviation.

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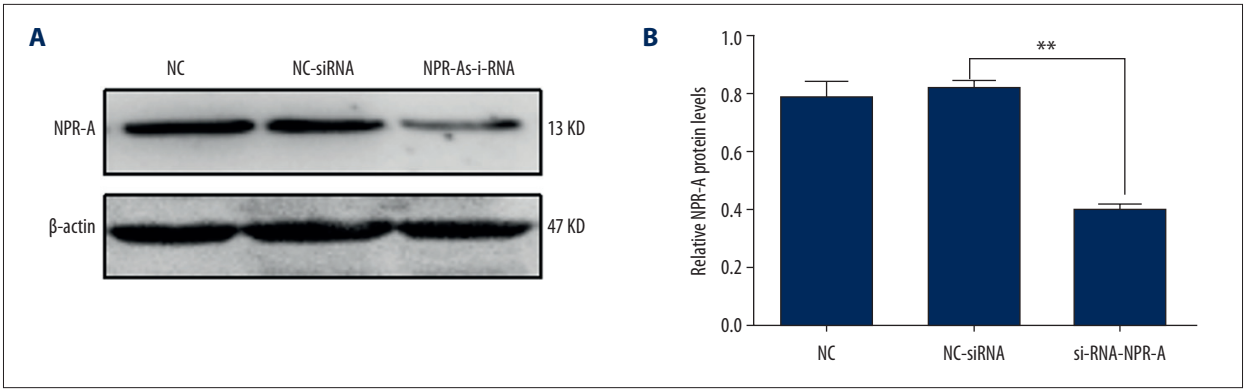

Figure 5. The transfection efficiency of NPR-A-siRNA. (**A**) The protein level was assessed by Western blot. (**B**) Statistical bar graphs of the Matrigel invasion assay. Relative NPR-A protein expression was assessed by densitometry analysis and normalized to β-actin. Data are presented as means (SD), ** P<0.01. NPR-A – natriuretic peptide receptor A; SD – standard deviation.

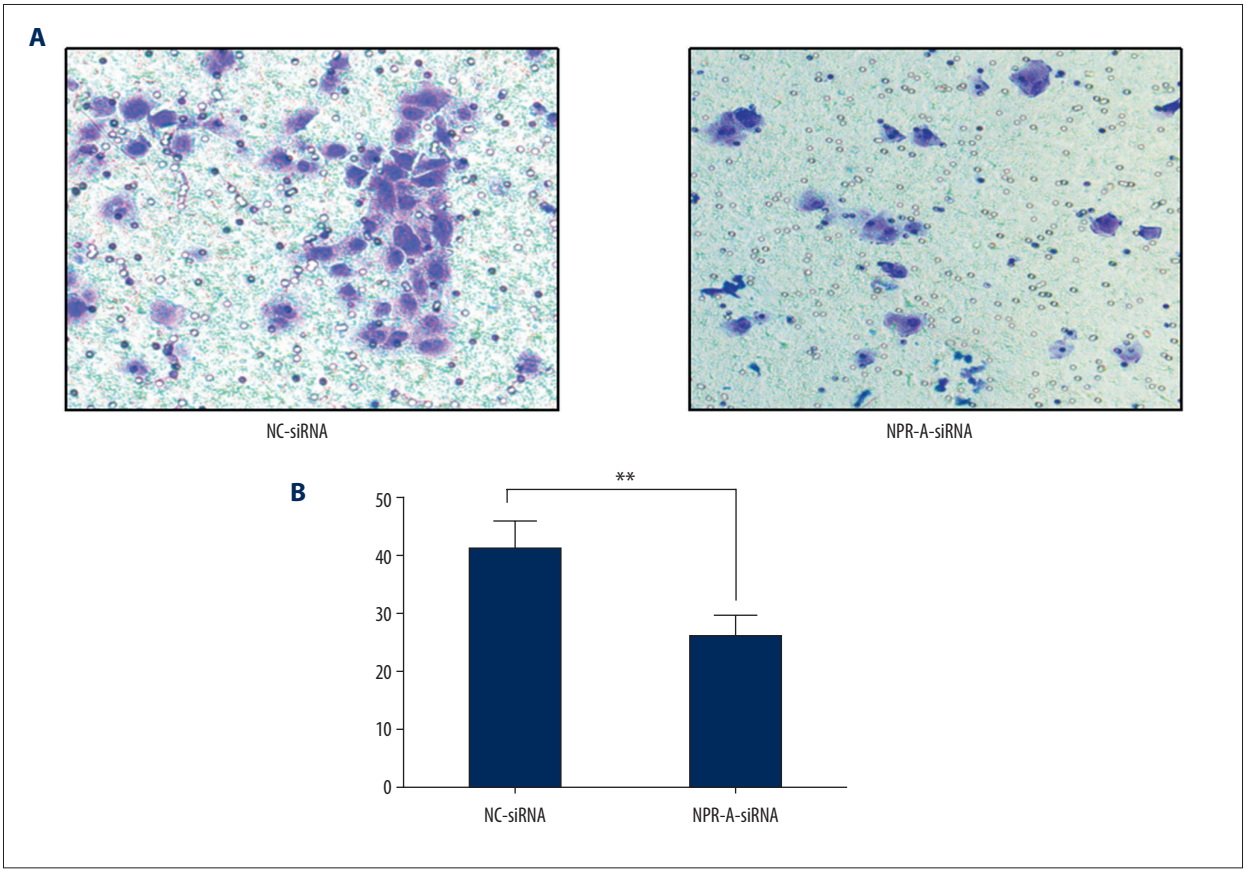

Figure 6. Effects of NPR-A knockdown on HTR8/SVneo invasion ability. (**A**) The invasive ability of HTR-8/SVneo cells with NPR-A knockdown (NPR-A-siRNA) was significantly lower than that of cells transfected with NC-siRNA. (**B**) Statistical bar graphs of the Matrigel invasion assay. Data are presented as means (SD), ** P<0.01. NPR-A – natriuretic peptide receptor A; SD – standard deviation.

NPR-A knockdown in human trophoblast cells (HTR-8/SVneo)

The aberrant decrease in NPR-A expression in PE placenta prompted us to explore the underlying mechanism of NPR-A. NPR-A-siRNA was transfected into HTR-8/SVneo cells, and NPR-A expression was measured by Western blot 48 h later. NPR-A-siRNA resulted in a 50% decrease in NPR-A levels compared to the negative control (P=0.01; Figure 5A, 5B).

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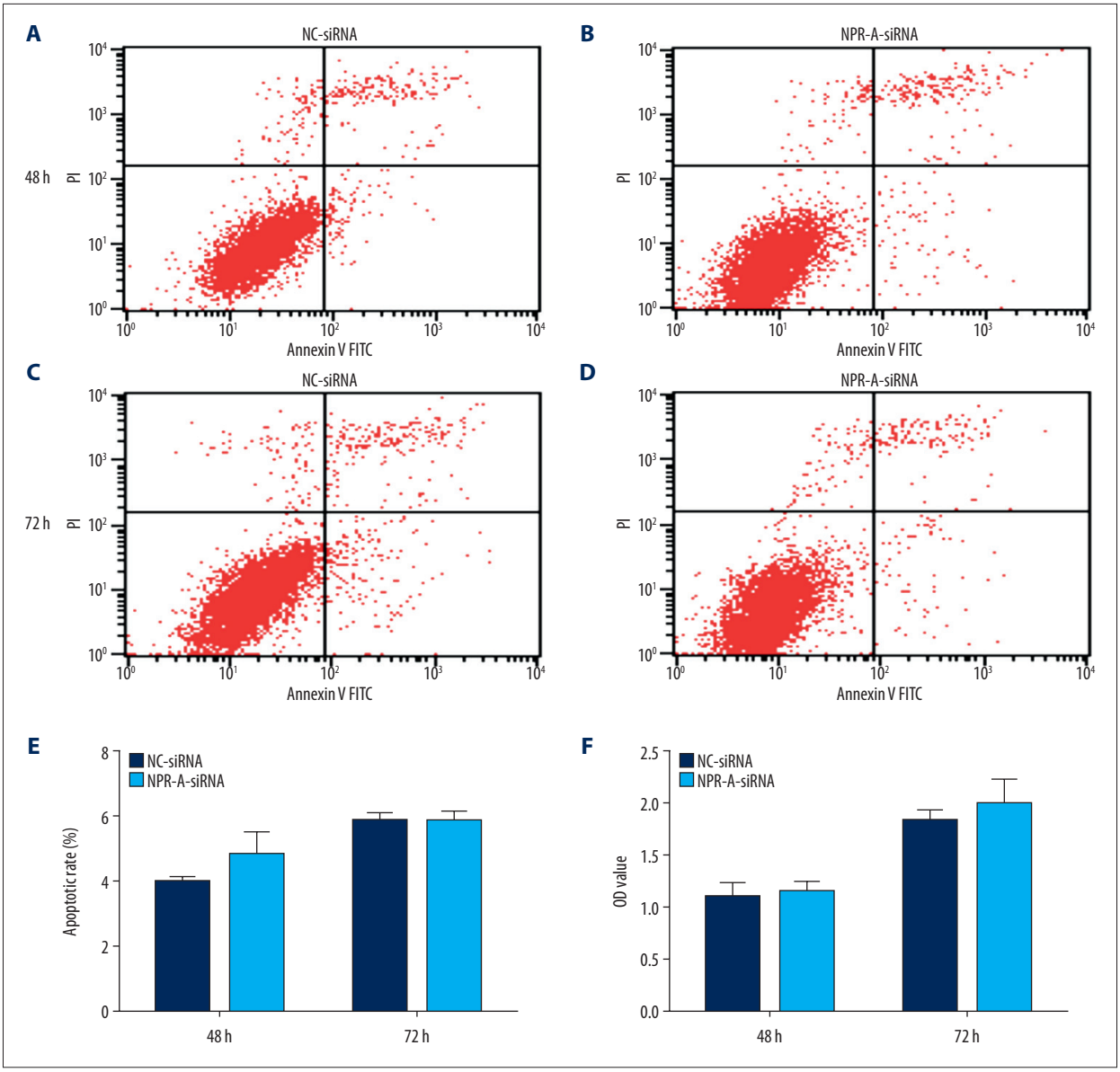

Figure 7. The effects of NPR-A knockdown on HTR8/SVneo cell apoptosis or proliferation. (**A–D**) Flow cytometry analysis (FCM analysis) was used to assess the effect of NPR-A on HTR-8/SVneo cells. There was no significant difference in apoptosis rates between HTR8/SVneo cells transfected with NPR-A siRNA for 48 h (**A, B**) or 72 h (**C, D**) and HTR8/SVneo cells transfected with NC-siRNA. (**E**) Statistical bar graphs of the flow cytometry analysis. Data are presented as means (SD). (**F**) CCK-8 assay was used to examine the effect of NPR-A on HTR-8/SVneo cell proliferation. There was no significant difference in apoptosis rates between HTR8/SVneo cells transfected with NPR-A siRNA for 48 h or 72 h and HTR8/SVneo cells transfected with NC-siRNA. Data are presented as means (SD). NPR-A – natriuretic peptide receptor A; SD – standard deviation; CCK-8 – Cell Counting Kit-8.

NPR-A knockdown impaired the invasion of HTR8/SVneo cells

NPR-A knockdown significantly impaired the invasion potential of HTR8/SVneo cells compared to those transfected with NC-siRNA (25.83±3.76 *vs.* 42.12±5.27, P=0.000; Figure 6).

NPR-A knockdown had no effect on HTR8/SVneo cell proliferation

At 48 and 72 h after transfection with NPR-A siRNA, the OD value of HTR8/SVneo cells was similar to that of cells transfected with NC-siRNA. This indicates that NPR-A downregulation has

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no significant effect on the proliferation of HTR8/SVneo cells (48 h: P=0.379; 72 h: P=0.139; Figure 7F).

NPR-A knockdown had no effect on HTR8/SVneo cell apoptosis

At 48 and 72 h after transfection with NPR-A-siRNA and NC-siRNA, apoptosis levels were determined by flow cytometry analysis. There was no difference in the apoptosis rate of the 2 cell groups (48 h: P=0.081; 72 h: P=0.061; Figure 7A–7E).

Discussion

The development of the human placenta is critical for embryonic development and a successful pregnancy outcome [16]. It has been shown that poor early placentation plays a central role in preeclampsia pathogenesis [17]. Previous studies with mice have documented that ANP and NPR-A are important in placental and embryonic development and PE pathogenesis in mice [12,13]. Aberrant forms of ANP and its receptors have been identified in patients with PE [8–11]. However, the role of ANP and NPR-A in the development of the human placenta and the pathogenesis of PE have not yet been clarified. As early as 1994, the expression of ANP and NPR-A was detected in human placenta and decidua using a radioimmunoassay or Northern blot analysis [18,19]. However, the localization of ANP and NPR-A in the placenta and decidua had not yet been elucidated. In this study, we used immunohistochemistry and found that ANP and NPR-A were expressed in the trophoblast subtypes of placental villi and decidua. ANP-positive and NPR-A-positive cells were abundantly clustered around and infiltrated into the vascular wall of the spiral artery undergoing remodeling. This result suggests that ANP and NPR-A may play a role in human placental development.

The conversion of the maternal decidual and myometrial vasculature by invasive trophoblasts is essential to placental development [20]. Further, EVT invasion is critically regulated by crosstalk between EVTs and decidual cells in a paracrine and autocrine manner. The endometrial glands in the decidua can secrete a variety of growth factors and other secretions that can modulate immune response, trophoblast invasion, and placental morphogenesis. Recent studies have shown that NPR-A is expressed at high levels in pre-implantation embryos and in undifferentiated murine ES [21,22]. Consistent with the above findings, in our study we found that ANP and NPR-A were localized in the glandular epithelium and EVTs in human decidua. *In vitro* experiments have shown that ANP can increase the invasion potential of BeWo cells (a choriocarcinoma cell line), possibly via NPR-A [12]. Studies in oncology have shown that NPR-A is abundantly expressed in several cancer cell lines and that it has an important role in cancer cell invasion. These findings indicate that ANP and NPR-A may be involved in regulating trophoblast function during human placental development.

In addition to EVTs, macrophages and uterine natural killer cells (uNKs) were found to infiltrate the vascular wall of spiral arterioles during spiral artery remodeling, prior to the presence of EVTs within the vessel lumen [23]. These cells can secrete many cytokines and enzymes, including matrix metalloproteinase (MMPs) and Ang II, and can disrupt the VSMCs, which are essential for complete spiral artery remodeling by invasive trophoblasts [24]. In the present study, we demonstrated that ANP-positive and NPR-A-positive cells were abundantly distributed throughout the decidual stroma, and particularly clustered around and infiltrated into the vascular wall of the spiral artery undergoing remodeling. Prior studies have found that ANP and its receptors are expressed in various immune organs and cells, including macrophages and natural killer cells, and that they are important in regulating the functions of macrophages and natural killer cells [25–29]. A recent study has demonstrated that ANP is expressed in mouse uNKs [30]. These results suggest a possible role of ANP and NPR-A in uterine spiral artery remodeling during placental development, which needs further clarification.

Placental dysfunction plays a central role in preeclampsia pathogenesis. Prior studies have shown that the affinity of ANP for its receptors was significantly decreased in preeclamptic placenta, and that ANP receptors were reduced in human placenta from growth-restricted fetuses, which shares the same pathogenesis as PE. Consistent with previous studies, we found that NPR-A expression was significantly decreased in placenta from patients with severe preeclampsia, suggesting that a reduction in NPR-A is associated with the underlying pathogenesis of PE. Nevertheless, the underlying signaling mechanism responsible for NPR-A downregulation in PE placenta is unclear. Studies in other cell lines have found that high levels of ANP, transforming growth factor-beta 1 (TGF-β1), and endothelin, which have also been identified in PE patients, can suppress NPR-A expression [31–35]. However, whether those factors are responsible for the downregulation of NPR-A expression in PE placenta, and what the underlying mechanism of downregulation is, still needs to be clarified.

As EVTs are central to placental development, we suspected that a reduction in NPR-A may contribute to the biological activities of EVTs, inducing PE. To further investigate the role of NPR-A in EVTs, we selectively knocked down NPR-A expression in an HTR8/SVneo cell line using an RNA interference approach, in order to evaluate its role in cell function. We found that NPR-A knockdown significantly impaired HTR8/SVneo cell invasion but had no effect on the proliferation and apoptosis of HTR8/SVneo cells. Studies in oncology have demonstrated that NPR-A is involved in tumorigenesis and tumor progression.

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It has been reported that NPR-A is highly expressed in several tumors, including lung carcinoma, melanoma, ovarian, prostate, and gastric cancer cells [36–39]. Data from animal models have shown that NPR-A deficiency may inhibit cancer cell growth and protect C57BL/6 mice from lung, skin, and ovarian cancer [37]. Further, *in vitro* results from various cancer cell lines have demonstrated that NPR-A downregulation can decrease the invasion and migration potential of tumor cells in many ways, including through the regulation of MMP2 and MMP9, which have been reported to exert a central role in trophoblast invasion [40]. NPR-A is now regarded as a novel target for cancer therapy. In agreement with recent studies on cancer cells, we found that NPR-A downregulation significantly decreased the invasion potential of HTR8/SVneo cells. This result further confirms the involvement of NPR-A in trophoblast invasion and placental development, and suggests that decreased NPR-A levels may contribute to the development of preeclampsia by regulating the invasion of trophoblast cells.

Apoptosis, an active process in the maintenance of normal functions, has been detected in the placenta throughout normal pregnancy [41,42]. However, a greater incidence of apoptosis has been detected in villous as well as extravillous trophoblasts from pregnancies complicated by PE, which suggests that dysregulated trophoblast apoptosis may be involved in PE pathogenesis [43-46]. Results from prostate cancer cell lines and human gastric cancer AGS cells have shown that NPR-A downregulation induced apoptosis, likely by decreasing the expression of macrophage migration inhibitory factor (MIF) and KCNQ1, a K^+ channel [37]. This study suggests that NPR-A plays an important role in the regulation of cell apoptosis. However, in our research, no effect of NPR-A on apoptosis was found in HTR8/SVneo cells, suggesting that NPR-A may take on different functions depending on the cellular context.

Conclusions

In conclusion, we examined the expression of ANP and NPR-A in human placenta and decidua and its role in the pathogenesis of preeclampsia. We found that ANP and NPR-A were expressed in the trophoblast subtypes of placental villi and decidua. ANP-positive and NPR-A-positive cells were abundantly clustered around and infiltrated into the vascular wall of the spiral artery undergoing remodeling. Moreover, NPR-A was decreased in the placenta of patients with severe preeclampsia compared to normal patients. Downregulation of NPR-A significantly reduced the invasion potential of HTR8/SVneo cells. These findings highlight the important role of ANP and NPR-A in human placental development and suggest that NPR-A downregulation may contribute to the development of preeclampsia by regulating the invasion of trophoblast cells.

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