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Chorionic villus-derived mesenchymal stem cell-mediated NRG1 upregulation promotes HTR-8/SVneo cells proliferation through the activation of the NF-κB signaling pathway

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ABSTRACT

In a prior study, our group found that chorionic villus-derived mesenchymal stem cells (CV-MSCs) were capable of promoting trophoblast proliferative and invasive activity. The mechanistic basis for this activity, however, has yet to be clarified. As such, an RNA-Seq analysis was conducted using trophoblasts that were treated with or without CV-MSC-conditioned media. Of the differentially expressed genes identified when comparing these two groups of cells, 23 proliferation-associated genes were identified and knocked down to test their functional roles in trophoblasts. These analyses revealed that inhibiting neuregulin 1 (NRG1) expression was sufficient to suppress proliferation and induce cell cycle arrest in trophoblasts. Placental samples from patients with preeclampsia exhibited significantly increased NRG1 expression relative to samples from healthy pregnancies. Following treatment with CV-MSC-conditioned media, NRG1 was upregulated in trophoblasts at the mRNA and protein levels. Relative to control trophoblasts, those in which NRG1 had been knocked down exhibited significantly impaired proliferation and DNA replication with the inactivation of the NF-κB signaling pathway. In contrast, overexpressing NRG1 yielded the opposite trophoblast phenotypes. Even in cells overexpressing NRG1, inhibition of NF-κB signaling was sufficient to significantly suppress trophoblast proliferation ($P < 0.05$). These results indicate that elevated NRG1 expression may play a role in the ability of CV-MSCs to induce proliferative activity in trophoblasts through the NF-κB signaling axis.

1. Introduction

Preeclampsia (PE) is a highly prevalent and serious disorder that affects 3–8% of all pregnancies and has the potential to cause severe maternal or fetal complications, including death [1]. PE manifests in the form of maternal hypertension and concomitant multi-organ dysfunction as a result of the aberrant endothelial function [2]. While average PE incidence rates have not changed in recent years, the total number of diagnosed cases continues to rise [3]. Mesenchymal stem cell (MSC)-based therapeutic interventions

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have been leveraged to treat many different diseases as these cells can be readily cultured and expanded *in vitro* while remaining minimally immunogenic [4–6]. In a prior study, our team discovered that human CV-MSCs were capable of enhancing the proliferative and invasive activity of trophoblasts through the activation of STAT3/JAK2 signaling [7]. However, further research is needed to fully elucidate these mechanisms through which CV-MSCs can alter trophoblast functionality.

Neuregulin (NRG) proteins are epidermal growth factor (EGF)-related molecules that are expressed at high levels in neurons and that play important roles in the development of many organ systems including the heart and nervous system [8]. NRG1 is capable of binding several ErbB family members including ErbB2, ErbB3, and ErbB4 [9]. NRG1 also reportedly plays a role as a driver of post-injury tissue repair, contributing to the functional recovery that occurs in injured tissues associated with the recruitment of MSCs, the induction of an immune response, and the regulation of the inflammatory microenvironment, and so on [10,11]. NRG1 also plays a role in CV-MSC-induced trophoblast functional recovery related to the pathogenesis of PE [12]. Accordingly, we hypothesized that the activation of NRG1/Erb2 signaling may contribute to the protective benefits that CV-MSCs confer to PE trophoblasts.

In our prior study was conducted an RNA-Seq analysis of trophoblasts that had been treated with CV-MSCs to identify important genes involved in the beneficial effects of such treatment. Through the silencing of 23 candidate genes in trophoblasts, we ultimately found that NRG1 knockdown could significantly reduce the proliferation of trophoblasts together with NF-κB signaling pathway inhibition. The present study was designed to follow up on these results by assessing the impact of changes in NRG1 expression induced by CV-MSCs on the proliferation of trophoblasts via the NF-κB signaling pathway.

2. Materials and methods

2.1. Cell culture

HTR8/SVneo trophoblast cells were obtained from the Type Culture Collection China Centre, and were cultured in DMEM/F12 (Gibco, CA, USA) containing 10% FBS and 1% penicillin/streptomycin in a 5% CO_2 incubator at 37 °C.

2.2. Conditioned medium preparation

CV-MSC-conditioned medium was prepared using previously established protocols [13]. Briefly, CV-MSCs were cultured to 80% confluence at which time the overlying media was discarded and cells were cultured for 24 h in serum-free DMEM/F12. Media was collected, centrifuged, and passed through a 0.22-μm filter (Millipore, MA, USA).

2.3. Cell transfection

HTR-8/SVneo cells were cultured for 24 h until subconfluent, at which time they were transiently transfected with NRG1-targeted siRNA constructs (with a non-targeting scramble control Hanbio, Shanghai, China) or overexpression plasmids using Lipofectamine 2000 (Invitrogen, USA).

The siRNA constructs used for this study were as follows: siNC, 5′- UUCUCCGAACGUGUCACGUTT -3’; reverse: 5′-ACGUGACACGUUCGGAGAATT-3’; siRNA-NRG1, 5′-GGUCUGAACGAAACAAUAUTT-3′, reverse: 5′-AUAUUGUUUCGUUCAGACCTT-3’. The NRG1 overexpression plasmids used for this study were as follows: 5′-ATGGGGAAAGGACGCGCGGGCCGAGTTGGC -3′, reverse: 5′-CTTTCACTATGAGGAGAGTCTCGGTA-3’.

2.4. qPCR

The fresh placental tissue were cut and grind broken fully in liquid nitrogen, total RNA was isolated from the placental tissue samples or cells using the Trizol Regent (Takara, Japan) according to the protocol. Then, a Reverse transcription kit (Invitrogen) was used to prepare cDNA, and qPCR was performed with SYBR Premix Ex Taq (Takara, Japan) and an ABI 7500 instrument. GAPDH served as a normalization control. All analyses were repeated in triplicate.

The primer sequences were as follows: NRG1, forward, 5′-GATCCTCACTCACCCTTCGC-3′, reverse, 5′-TAACCACACAGGGAGTGTGC-3’; TAZ, forward, 5′- CTCACATCCTGGCGACTCTC-3′, reverse, 5′- CACGAGCTAGGCTTCGGATT-3’; GAPDH forward, 5′- AATGGGCAGCCGTTAGGAAA-3′, reverse, 5′- GCGCCCAATACGACCAAATC-3’.

2.5. Immunohistochemistry

Placental tissues from PE patients (n = 5) and normal full-term pregnancies (n = 5) after cesarean section were subject to 45-min fixation. The tissues were then embedded in paraffin, sliced into 4-μm sections, and deparaffinized. Then, the slides were boiled in 6.0-pH sodium citrate buffer for 7 min. Endogenous peroxidase was blocked by hydrogen peroxide for 10 min. Each slide was washed with TBS for 5 min three times. Next, these slides were incubated with anti-NRG1 antibodies (1:5,00) at 4 °C overnight. After treated with diluted biotinylated secondary antibodies, the target proteins were visualized by DAB stain. Haematoxylin was subsequently used for counterstaining. The NRG1 expression was assessed by optical microscope. The NRG1 is mainly expressed in the cytoplasm of extravillous trophoblasts outside the placenta villi, and its expression in PE patients were decreased significantly.

2.6. Western immunoblotting

Cells were lysed with chilled RIPA buffer (Sigma-Aldrich, MO, USA). Samples were centrifuged (12,000×*g*), and lysates were added to LDS sample buffer. Samples were separated via SDS-PAGE and transferred to PVDF membranes (Bio-Rad, CA, USA) that were then blocked using 5% nonfat milk, incubated overnight with primary antibodies at 4 °C overnight, and incubated with a secondary antibody (1:1000; CST, MA, USA). Protein was then detected with a chemiluminescent detection system (Bio-Rad).

Primary antibodies used in this study were specific for the following: NRG1 (ab 191139, Abcam, MA; 1:500), ErbB2 (ab 241325, Abcam; 1:1000), *p*-Akt (ab 241325, Abcam; 1:1000), *p*-ERK (ab229912, Abcam; 1:1000), *p*-mTOR (ab 109268, Abcam; 1:500), GAPDH (ab181603, Abcam; 1:2000), p-P65(ab 53489, Abcam; 1:1000), P65 (ab 32536, Abcam; 1:5000), LaminB (ab 16048, Abcam; 1:1000).

2.7. EdU assay

An EdU proliferation kit (RiboBio, Guangzhou, China) was used based on provided instructions to assess cell proliferation based on the proportion of EdU-positive trophoblasts to the total number of cells. The trophoblasts were treated with CM for 24 h and tested by EdU kit immediately. Furthermore, EdU assays were performed 24 h after cell transfection. All analyses were repeated in triplicate.

2.8. Cell cycle analyses

The trophoblasts were treated with CM for 24 h, and the going through overnight serum starvation to synchronize cell cycle progression. Cell cycle analyses were conducted using an appropriate detection kit (Keygen Biotech). Briefly, trophoblasts were harvested, rinsed using PBS, and fixed overnight with 70% ethanol at 4 °C. After an additional wash with PBS, cells were stained at room temperature for 30 min using propidium iodide (PI)/RNase (500 μl) while protected from light. A BD LSR Fortessa flow cytometer was then used to analyze cell cycle progression. All analyses were repeated in triplicate.

2.9. Statistical analysis

Data are means ± SD from three or more independent experiments and were compared via two-tailed unpaired Student's t-tests or one-way analysis of variance (ANOVA). Data were analyzed with GraphPad Prism (GraphPad, CA, USA). $P < 0.05$ was the significance threshold.

3. Results

NRG1 is expressed at lower levels in PE placenta tissues and upregulated in HTR-8/SVneo cells response to CV-MSCs.

To explore the mechanisms whereby CV-MSCs can alter trophoblast functionality, we re-analyzed previously generated RNA-seq data to identify differentially expressed genes (DEGs) in CV-MSC-treated trophoblasts (no. SRR9943697-9943702, SRA). In total, 23 DEGs (related to the cell cycle and proliferation, listed Table 1), including NRG1, were selected and individually knocked down using

Table 1
Selected DEGs.

	gene_id	log2FoldChange	padj
HSPA6	ENSG00000173110	−9.664314194	1.11E-05
NRG1	ENSG00000157168	−4.615773987	1.95E-70
FN1	ENSG00000115414	−4.182690812	2.14E-48
COL5A3	ENSG00000080573	−4.025173781	2.59E-08
MKX	ENSG00000150051	−3.588054881	0.000249098
RNF150	ENSG00000170153	−3.503721998	3.16E-13
AMIGO2	ENSG00000139211	−3.409644109	2.73E-66
VSTM1	ENSG00000189068	−3.322446864	0.003653498
ADAMTS1	ENSG00000154734	−3.290074959	6.13E-11
RHOJ	ENSG00000126785	−3.137236275	1.98E-05
ASB5	ENSG00000164122	−2.791028158	3.55E-05
RASSF6	ENSG00000169435	2.848905387	0.04282923
RASD2	ENSG00000100302	2.994993142	2.20E-18
SMAD6	ENSG00000137834	3.011363862	3.49E-05
NOG	ENSG00000183691	3.144362489	1.77E-14
COL13A1	ENSG00000197467	3.290037837	5.34E-34
ENPP2	ENSG00000136960	3.426112428	9.70E-16
ATOH8	ENSG00000168874	3.495040321	9.66E-07
KRT81	ENSG00000205426	3.780895166	5.82E-12
ID1	ENSG00000125968	4.067674517	5.67E-29
CGB8	ENSG00000213030	4.276973879	9.69E-06
NPTX1	ENSG00000171246	4.711454883	1.96E-10
TXNIP	ENSG00000265972	5.367444904	4.27E-05

siRNA constructs to assess changes in trophoblast cell cycle progression. In this analysis, the knockdown of NRG1 was found to significantly reduce the frequency of G1 phase trophoblasts. Relative to placental tissue from healthy pregnancies, placental tissue from women with PE (information in Table 2) exhibited significantly lower mRNA and protein levels of NRG1 expression (Fig. 1A–B). Treatment with CV-MSC-conditioned medium was sufficient to significantly upregulate NRG1 in HTR-8/SVneo cells (Fig. 1C–D). Together, these results thus suggest that NRG1 expression in trophoblasts could be regulated by CV-MSC treatment.

3.1. NRG1 knockdown suppresses DNA synthesis and inactivates NF-κB signaling in trophoblasts

Initially, qPCR analyses revealed that the expression of NRG1 in HTR8/SVneo cells was effectively suppressed by siRNA transfection (Fig. 2A). EdU and cell cycle assays were then used to assess the functional role of NRG1 in these cells. In EdU uptake assays, NRG1 knockdown was found to suppress DNA synthesis in these trophoblasts (Fig. 2B). In line with these results, knocking down NRG1 lowered the frequency of cells in the G1 phase while increasing the frequency of G2/M phase cells (Fig. 2C). It has been well-documented that overexpression of ErbB2 causes NF-κB activation, which involved PI3K/Akt pathway. ErbB2 first activates the PI3K/Akt pathway (one of the most important pathways for cell growth, proliferation, and survival) for NF-κB activation [14]. So we examined *p*-Akt, *p*-ERK, *p*-mTOR and P65 expression to understand ErbB2 mediated PI3K/Akt–NF–κB activation. Moreover, the activation of NF-κB pathway was observed in GSEA assays (data not shown). Western immunoblotting additionally revealed that PI3K/Akt–NF–κB signaling was inactivated in these trophoblasts in which NRG1 had been silenced (Fig. 2D). Together these data suggest that CV-MSCs can upregulate NRG1 in trophoblasts, potentially thereby controlling trophoblast proliferation via the regulation of NF-κB pathway signaling.

3.2. Overexpressing NRG1 enhances DNA synthesis in trophoblasts and promotes NF-κB pathway activation

To more fully understand how NRG1 mediates the effects of CV-MSCs on the proliferative activity of exposed trophoblasts, HTR8/SVneo cells overexpressing NRG1 were next established (Fig. 3A). In EdU uptake assays, the overexpression of NRG1 was found to enhance trophoblast DNA synthesis activity (Fig. 3B). NRG1 also altered cell cycle progression in these cells (Fig. 3C). In Western immunoblotting assays, overexpressing NRG1 was also found to promote NF-κB pathway activation in these trophoblasts (Fig. 3D). Together these findings suggested that in trophoblasts, NRG1 is capable of promoting DNA synthesis and activating NF-κB signaling, potentially serving as a mediator of CV-MSC-induced trophoblast proliferation.

3.3. NRG1 controls the synthesis of DNA in trophoblasts through the NF-κB signaling pathway

To support the above findings, the role that NF-κB signaling plays as a mediator of NRG1-driven trophoblast proliferation was analyzed by treating these cells with the NF-κB inhibitor triptolide [15]. Triptolide is a diterpene trioxide extracted from *Tripterygium wilfordii* Hook F, and several prior reports have demonstrated its ability to effectively and efficiently inhibit NF-κB pathway activation [15,16]. Accordingly, trophoblasts overexpressing NRG1 were treated with triptolide (0.5 pM), after which EdU uptake and cell cycle progression were analyzed (Fig. 4A–B). Consistent with the above data, inhibiting NF-κB significantly reduced DNA synthesis in these trophoblasts, lowering cellular viability in these NRG1-overexpressing trophoblasts below the levels observed in cells expressing normal NRG1 levels. Lastly, Western immunoblotting assays were conducted, confirming that NRG1 overexpression and triptolide treatment altered NRG1 expression and NF-κB associated markers in these trophoblasts (Fig. 4C). To further confirm the NF-κB signalling activation of trophoblasts, we also tested the p-P65 and P65 expression in trophoblasts nuclear and cytoplasm (Fig. 4D). The results showed that NRG1 overexpression could increase the p-P65 level in trophoblasts and P65 expression in trophoblasts nuclear, which indicated the NRG1 could activate the NF-κB signalling.

Overall, these findings confirm a role for NRG1 as a mediator of the CV-MSC-induced proliferation of trophoblasts through the regulation of NF-κB signaling activity.

Table 2
Placental tissue from women with PE.

	Number	age	Gravidity and parity	delivery	fetal sex	fetal weight	placental weight	Gestational age
vector	1	32.23	G2P1	eutocia	girl	3.200 KG	0.600 KG	37.62W
	2	30.45	G1P1	cesarean	girl	3.160 KG	0.550 KG	38.42W
	3	28.26	G3P1	cesarean	girl	3.800 KG	0.600 KG	38.14W
	4	31.77	G2P1	eutocia	boy	3.200 KG	0.620 KG	37.29W
	5	28.16	G1P1	eutocia	girl	3.040 KG	0.650 KG	37.57W
PE	1	30.26	G2P2	cesarean	girl	1.820 KG	0.350 KG	36.00W
	2	28.67	G1P1	cesarean	girl	2.020 KG	0.380 KG	36.62W
	3	32.76	G3P1	cesarean	boy	1.890 KG	0.320 KG	37.42W
	4	27.12	G1P1	cesarean	girl	1.960 KG	0.370 KG	36.86W
	5	26.56	G2P1	cesarean	boy	2.170 KG	0.400 KG	37.14W

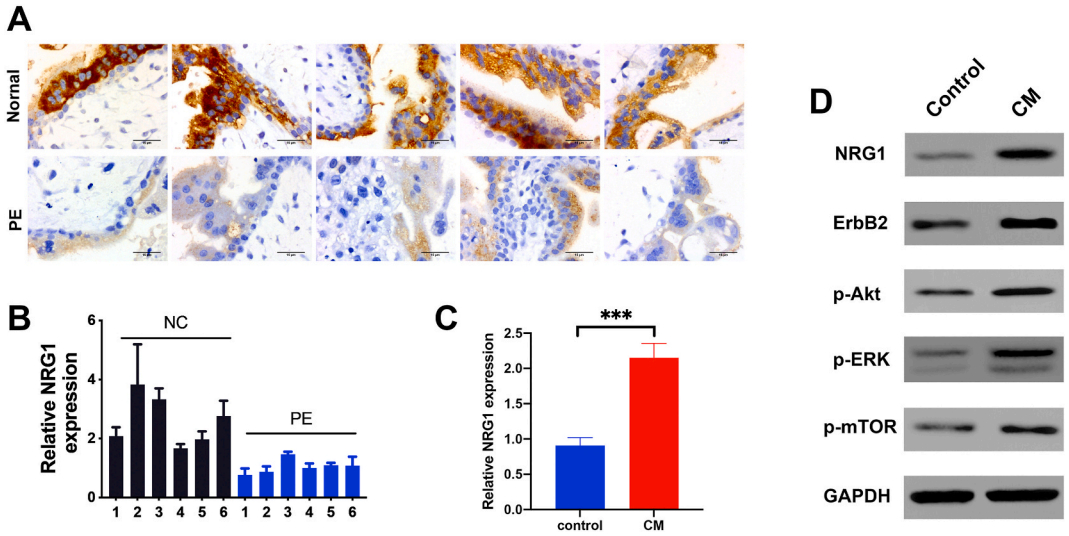

Fig. 1. NRG1 is downregulated in PE placental tissue and upregulated in trophoblasts in response to CV-MSCs. (A) Placental tissue samples from PE patients and healthy controls were subject to staining to detect NRG1 expression. (B) NRG1 mRNA expression was analyzed in placental tissue samples from PE patients and healthy controls via qPCR. (C, D) NRG1 expression was compared in trophoblasts that had or had not been treated with CV-MSCs at the mRNA (C) and protein (D) levels by qPCR and Western immunoblotting, respectively. GAPDH served as a normalization control. Student's t-test: $^{**}P < 0.01$, $^{***}P < 0.001$.

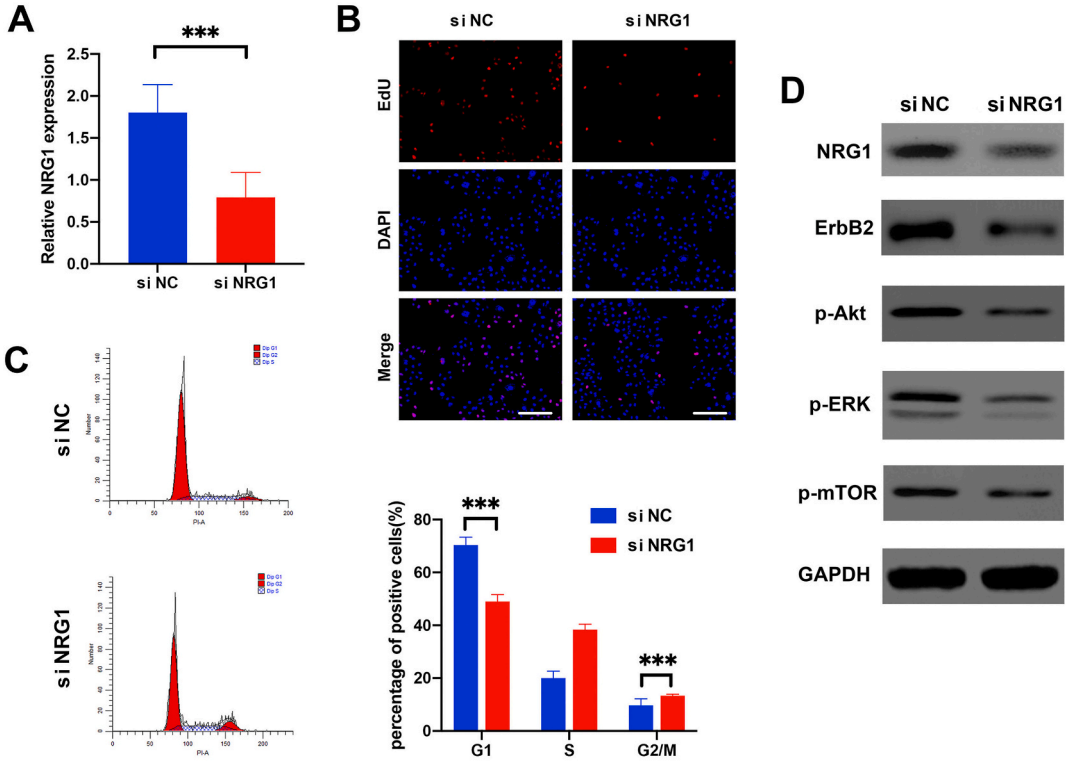

Fig. 2. NRG1 knockdown suppresses DNA synthesis and inactivates NF-κB signaling in trophoblasts. (A) NRG1 levels were analyzed in trophoblasts transfected with shNRG1 via qPCR. (B) Representative results of an EdU uptake assay in which EdU uptake (red) was used to detect dividing trophoblasts transfected with shNRG1, while DAPI (blue) was used to counterstain all cells. (C) Representative flow cytometry plots corresponding to the cell cycle distributions of trophoblasts following shNC or shNRG1 transfection. (D) NRG1 and NF-κB pathway-related protein expression was detected in transfected trophoblasts via Western immunoblotting. GAPDH served as a normalization control. Student's t-test: $^{**}P < 0.01$, $^{***}P < 0.001$.

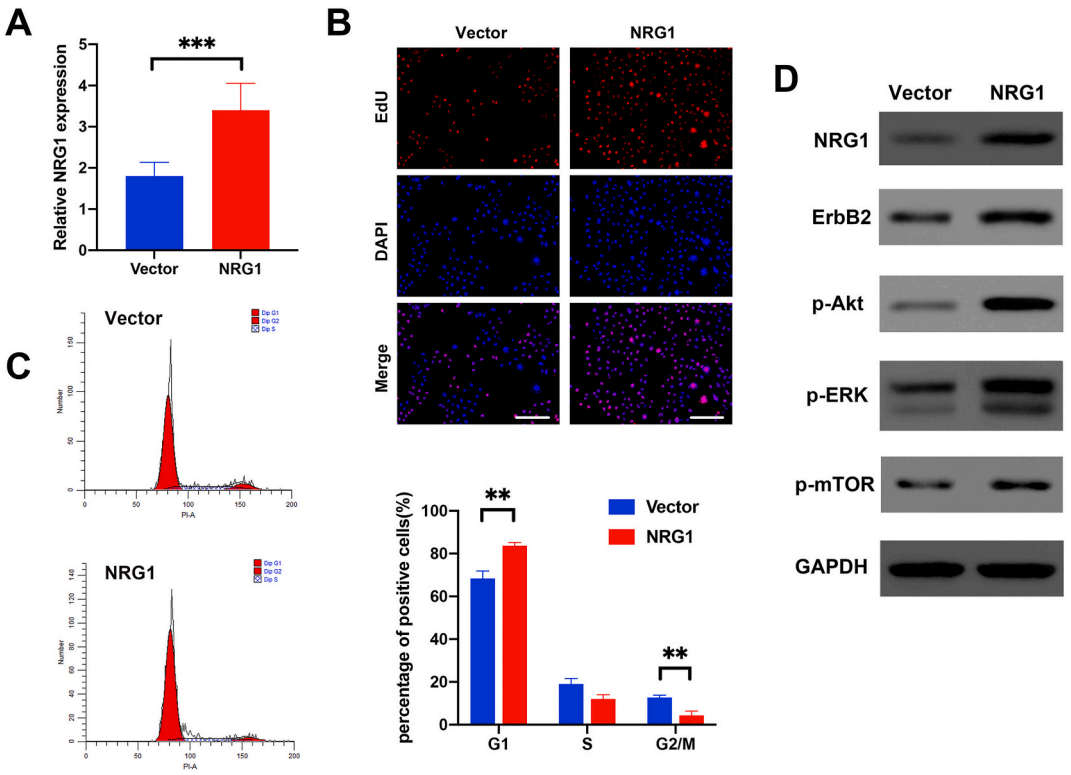

Fig. 3. Overexpressing NRG1 enhances DNA synthesis in trophoblasts and promotes NF-κB pathway activation (A) NRG1 overexpression in trophoblasts was confirmed at the mRNA level by qPCR. (B) Representative EdU uptake assay results for trophoblasts overexpressing NRG1 in which EdU uptake (red) was used to detect proliferating trophoblasts while DAPI (blue) was used to counterstain all cells. (C) Representative flow cytometry plots corresponding to the cell cycle distributions of trophoblasts overexpressing NRG1. (D) NRG1 and NF-κB pathway-related protein expression was detected in transfected trophoblasts via Western immunoblotting. GAPDH served as a normalization control. Student's t-test: $^{**}P < 0.01$, $^{***}P < 0.001$.

4. Discussion

Preeclampsia is a potentially serious pregnancy-related disorder that can expose both the mother and the fetus to high levels of risk, and delivery is the only known effective treatment for this disease [17,18]. PE can also contribute to higher long-term cardiovascular disease and diabetes incidence rates [19]. The pathogenesis of PE is driven in large part by inflammation and oxidative stress [20]. MSCs reportedly exhibit a range of beneficial effects *in vitro* and *in vivo* through various immunoregulatory, antioxidant, angiogenic, and anti-inflammatory mechanisms [21]. For example, MSC-based treatment can provide therapeutic benefits in preclinical heart, lung, liver, and kidney disease model systems [22]. Even so, there have been relatively few studies exploring the therapeutic effects of the MSC-based treatment of PE. In one recent report, MSC-based therapy was found to alleviate the symptoms of PE in a preclinical model system [23]. Additional work, however, will be vital to understand the pathogenic mechanisms that underlie PE development and the pathways through which MSCs can alleviate this disease.

CV-MSCs can be extracted from placental tissue and exhibit immunomodulatory activity and the ability to self-renew and undergo multipotent differentiation [24]. As they can exert antioxidant, anti-inflammatory, and immunoregulatory properties, CV-MSCs are often explored for MSC-based therapeutic treatment [25]. However, further research is required to more fully understand the regulatory functions of these cells in detail. In our prior study, we found that CV-MSCs were capable of promoting the proliferative and invasive activity of trophoblasts [7]. In this research, the mechanism of CV-MSCs promoting effects in trophoblasts was further studied. Our data showed that NRG1 was downregulated in trophoblasts of PE placenta than normal placenta, and CV-MSCs could increase the NRG1 levels in trophoblasts. Moreover, we found a strong correlation between upregulation of NRG-1 and increase proliferation in trophoblasts, which could mediated the therapeutic effects of CV-MSCs. We also identified NF-κB signaling as a potentially effective NRG1 downstream pathways.

Members of the NRG protein family (NRG1-4) function as growth factors that can bind to EGF receptors [26]. NRG1 reportedly plays essential physiological roles in the breast and heart tissue, and it has also been linked to the pathogenesis of cancer and other diseases [27,28]. When NRG1 binds to its cognate receptor, this leads to ErbB phosphorylation and triggers downstream PI3K/AKT/MAPK signaling activity [29]. NRG1 fusions are often detected in a range of cancers such that targeting this pathway may be a viable antitumor treatment strategy [27,30]. The importance of NRG1 as a mediator of PE pathogenesis was not fully understand,

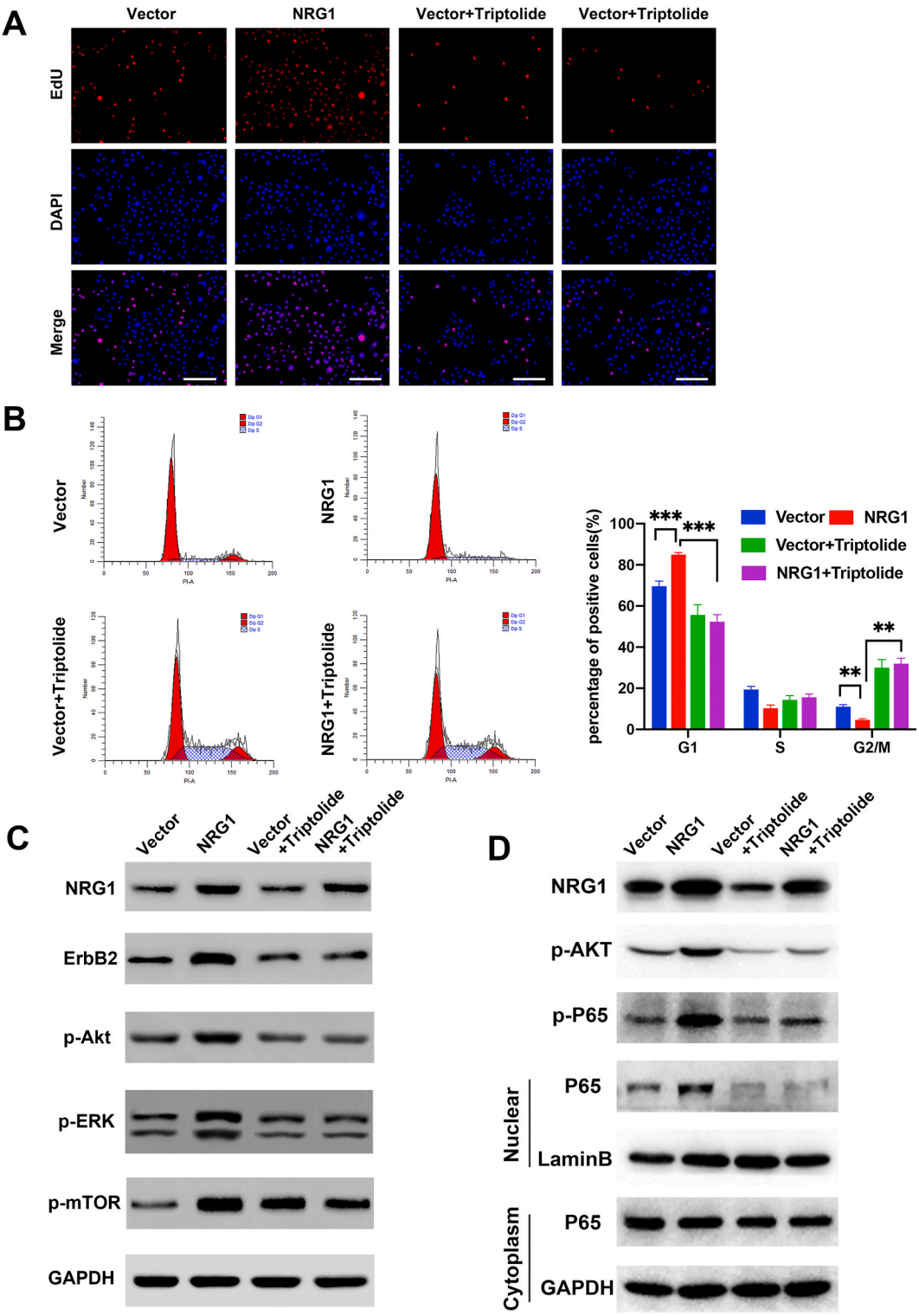

Fig. 4. NRG1 controls the synthesis of DNA in trophoblasts through the NF-κB signaling pathway. (A) Representative EdU uptake assay results for trophoblasts overexpressing NRG1 that had been treated with the NF-κB pathway inhibitor triptolide (0 or 0.5 pM). (B) Representative flow cytometry plots corresponding to the cell cycle distributions of trophoblasts overexpressing NRG1 that had or had not been treated with triptolide. (C) NRG1 and NF-κB signaling proteins were detected in transfected trophoblasts that had or had not been treated with triptolide via Western immunoblotting. (D) NRG1, p-P65, P65 in nuclear and in cytoplasm protein expression was detected in transfected trophoblasts that had or had not been treated with triptolide via Western immunoblotting. Student's t-test: $^{**}P < 0.01$, $^{***}P < 0.001$.

and our data showed that NRG1 was downregulated in PE trophoblasts and could be upregulated by CV-MSCs. Our observation indicated that the mechanism of decreased NRG1 expression in PE could rescued by CV-MSCs treatment was a potential therapeutic target.

Many reports have demonstrated a role for NRG1-ErbB signaling as a mediator of the therapeutic efficacy of adipose- or bone marrow-derived MSCs [31]. When NRG1 is applied exogenously, for example, this has been shown to improve the therapeutic outcomes associated with MSC treatment through NRG1-ErbB signaling activation [31,32]. Prior work from our group has also demonstrated that CV-MSCs can exert beneficial therapeutic effects when used to treat trophoblasts [7], and NRG1 upregulation has been detected in CV-MSC-treated trophoblasts. In this study, NRG1 was identified as one of 23 CV-MSC treatment-related DEGs that was able to regulate cell cycle progression in trophoblasts. Moreover, NRG1 overexpression in these trophoblasts led to the activation of NF-κB signaling pathway activity as was observed following CV-MSC treatment. Notably, knocking down or overexpressing NRG1 in these trophoblasts was sufficient to enhance or suppress ErbB2-related NF-κB signaling, thus modulating associated DNA synthesis in these trophoblasts. We then realized that functional impact of NRG1 dependent DNA synthesis and proliferation was closely related to the activation of NF-κB signaling. Several pieces of evidence confirmed that inactivation of NF-κB negated the promoting effects of CV-MSCs on trophoblasts.

In summary, our results provide clear support for this CV-MSCs/NRG1/NF-κB regulatory axis and its potential relevance in the context of PE. Future research focused on NRG1-ErbB-related signaling factors may lead to the discovery of novel alternative molecular alternative approaches to the CV-MSC-based treatment of PE.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Qingdao University Affiliated Hospital. All participants provided a written informed consent (QYFY WZLL 25511).

Authors' contributions

Y.Y. and Y.L. conceived and designed the experiments. Y.C., Y.L. and Y.W. performed the experiments. N.Z., J.Z., J.W and Y.L. analyzed and interpreted the data. Y.L. wrote the paper.

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Data availability

The data that support the findings of this study are included within the article and openly available in Sequence Read Archive (SRA) labelled as no. SRR11492552-SRR11492557.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e18245.

References

[1] J.F.B. Lambert, G. Hartstein, V. Bonhomme, P.Y. Dewandre, Preeclampsia: an update, Acta Anaesthesiol. Belg. 65 (4) (2014) 137–149.
[2] E.A. Phipps, et al., Pre-eclampsia: pathogenesis, novel diagnostics and therapies, Nat. Rev. Nephrol. 15 (5) (2019) 275–289.
[3] C.V. Ananth, K.M. Keyes, R.J. Wapner, Pre-eclampsia rates in the United States, 1980-2010: age-period-cohort analysis, BMJ 347 (2013) f6564.
[4] H. Tsuda, et al., Transplantation of allogenic fetal membrane-derived mesenchymal stem cells protects against ischemia/reperfusion-induced acute kidney injury, Cell Transplant. 23 (7) (2014) 889–899.

[5] F. Togel, et al., Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms, Am. J. Physiol. Ren. Physiol. 289 (1) (2005) F31–F42.
[6] R. Al-Rifai, et al., In vivo efficacy of endothelial growth medium stimulated mesenchymal stem cells derived from patients with critical limb ischemia, J. Transl. Med. 17 (1) (2019) 261.
[7] Y. Chu, et al., Chorionic villus-derived mesenchymal stem cell-mediated autophagy promotes the proliferation and invasiveness of trophoblasts under hypoxia by activating the JAK2/STAT3 signalling pathway, Cell Biosci. 11 (1) (2021) 182.
[8] X. Wang, et al., NFKB2 inhibits NRG1 transcription to affect nucleus pulposus cell degeneration and in fl ammation in intervertebral disc degeneration, Mech. Ageing Dev. 197 (2021), 111511.
[9] P. Christopher, J. Blomberg, The developing role of Neuregulin1 in cardiac regenerative stem cell therapy, Curr. Pharmaceut. Des. 20 (30) (2014) 4906–4910.
[10] T. Jarde, et al., Mesenchymal niche-derived neuregulin-1 drives intestinal stem cell proliferation and regeneration of damaged epithelium, Cell Stem Cell 27 (4) (2020) 646–662 e7.
[11] X. Liang, et al., Activation of NRG1-ERBB4 signaling potentiates mesenchymal stem cell-mediated myocardial repairs following myocardial infarction, Cell Death Dis. 6 (2015) e1765.
[12] S. Jin, et al., The pathological and therapeutic roles of mesenchymal stem cells in preeclampsia, Front. Med. 9 (2022), 923334.
[13] Y. Chu, et al., Co-culture with chorionic villous mesenchymal stem cells promotes endothelial cell proliferation and angiogenesis via ABCA9-AKT pathway, Faseb. J. 36 (10) (2022), e22568.
[14] Christina K. Galang, Patricia A. Solski, John K. Westwick, J. Der Channing, Nickolay N. Neznanov, Robert G. Oshima, Craig A. Hauser, Oncogenic neu/ErbB-2 increases ets, AP-1, and NF-kB-dependent gene expression, and inhibiting ets activation blocks neu-mediated cellular transformation, J. Biol. Chem. 271 (14) (1996) 1992–1998.
[15] W.Y. Fang, et al., Triptolide prevents LPS-induced skeletal muscle atrophy via inhibiting NF-kappaB/TNF-alpha and regulating protein synthesis/degradation pathway, Br. J. Pharmacol. 178 (15) (2021) 2998–3016.
[16] Q. Wan, et al., Triptolide ameliorates fine particulate matter-induced podocytes injury via regulating NF-kappaB signaling pathway, BMC Mol Cell Biol 21 (1) (2020) 4.
[17] N.B. de Alwis, N.K. Beard, S. Kaitu'u-Lino, T.J. Tong, S. Brownfoot, F. Hannan, Novel approaches to combat preeclampsia: from new drugs to innovative delivery, Placenta 102 (2020) 10–16.
[18] T.M. Tomimatsu, K. Matsuzaki, S. Endo, M. Kumasawa, K. Kimura, Preeclampsia: maternal systemic vascular disorder caused by generalized endothelial dysfunction due to placental antiangiogenic factors, Int. J. Mol. Sci. 20 (17) (2019).
[19] A.A. Karatza, G. Dimitriou, Preeclampsia emerging as a novel risk factor for cardiovascular disease in the offspring, Curr. Pediatr. Rev. 16 (3) (2020) 194–199.
[20] S. Gumusoglu, et al., The serotonin-immune Axis in preeclampsia, Curr. Hypertens. Rep. 23 (7) (2021) 37.
[21] J. Vasanthan, et al., Role of human mesenchymal stem cells in regenerative therapy, Cells 10 (1) (2020).
[22] D.E. Rodriguez-Fuentes, et al., Mesenchymal stem cells current clinical applications: a systematic review, Arch. Med. Res. (2020).
[23] S. Suvakov, et al., Emerging therapeutic potential of mesenchymal stem/stromal cells in preeclampsia, Curr. Hypertens. Rep. 22 (5) (2020) 37.
[24] Q. Huang, et al., An efficient protocol to generate placental chorionic plate-derived mesenchymal stem cells with superior proliferative and immunomodulatory properties, Stem Cell Res. Ther. 10 (1) (2019) 301.
[25] Y. Li, et al., Chorionic villus-derived mesenchymal stem cells induce E3 ligase TRIM72 expression and regulate cell behaviors through ubiquitination of p53 in trophoblasts, Faseb. J. 35 (12) (2021), e22005.
[26] B.K. Chun Fang, Zhao Pan, Ran Jing, Lifang Wang, Targeting neuregulin 1 (NRG1): a novel biomarker for non-small-cell lung cancer, J. Environ. Pathol. Toxicol. Oncol. 40 (4) (2021) 61–72.
[27] K.D. Howarth, et al., NRG1 fusions in breast cancer, Breast Cancer Res. 23 (1) (2021) 3.
[28] C. Birchmeier, D.L. Bennett, Neuregulin/ErbB signaling in developmental myelin formation and nerve repair, Curr. Top. Dev. Biol. 116 (2016) 45–64.
[29] N. Ptakova, et al., Identification of tumors with NRG1 rearrangement, including a novel putative pathogenic UNC5D-NRG1 gene fusion in prostate cancer by data-drilling a de-identified tumor database, Genes Chromosomes Cancer 60 (7) (2021) 474–481.
[30] S. Jonna, et al., Detection of NRG1 gene fusions in solid tumors, Clin. Cancer Res. 25 (16) (2019) 4966–4972.
[31] X.M. Yi, Y. Chen, G.J. Tu, Neuregulin1 impacting bone marrow mesenchymal stem cell migration is conducive to functional recovery following spinal cord injury, Mol. Med. Rep. 20 (1) (2019) 41–48.
[32] S. Ryu, et al., Therapeutic efficacy of neuregulin 1-expressing human adipose-derived mesenchymal stem cells for ischemic stroke, PLoS One 14 (9) (2019), e0222587.