The involvement of eukaryotic initiation factor 5A in trophoblast cell function

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Abstract

Abnormal growth and migration of trophoblast cells is one of the main causes of spontaneous abortion. Eukaryotic translation initiation factor 5A (eIF5A) plays an important role in trophoblast cell growth and migration; however, its underlying mechanism remains largely unknown. Here, we first confirmed that eIF5A knockdown reduced human chorionic trophoblast HTR8 cells viability, proliferation, and migration. Next, we sought to systematically identify the genes regulated by eIF5A and observed changes in the transcriptome profile of eIF5A-knockdown HTR8 cells by RNA-seq analysis. Especially, we found that inhibition of eIF5A reduced both the mRNA and protein levels of methyltransferase-like protein 14 (METTL14). Furthermore, inhibition of METTL14 expression resulted in the reduction of viability, proliferation, and migration of HTR8 cells. In addition, we showed that overexpression of METTL14 rescued the effects of eIF5A knockdown in HTR8 cells. Finally, we revealed that eIF5A and METTL14 expression was decreased in spontaneous abortion samples compared to that in elective-induced abortion samples. Collectively, our study demonstrated that eIF5A plays a crucial role in HTR8 cells via modulation of METTL14 expression and may serve as a novel potential target for spontaneous abortion diagnosis and treatment.

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Introduction

Human pregnancy is a complex biological process, and spontaneous abortion is the most common adverse pregnancy outcome (Hopfner *et al.* 2012). Studies have reported that nearly one in four women experience at least one spontaneous abortion in their lifetime, indicating a high incidence rate (Van *et al.* 2014). According to recent statistics, 31% of the human embryos undergo spontaneous abortion soon after implantation, 80% of which occur in early pregnancy (Rand 1993). Several pathogenic factors, including hereditary factors, endocrine disorders, and structural abnormalities of the reproductive system contribute to spontaneous abortions (Du *et al.* 2018). However, its etiology has not yet been fully elucidated. Therefore, further studies on the pathogenesis of spontaneous abortion as well as strategies to improve pregnancy outcomes are required for the prevention, diagnosis, and treatment of spontaneous abortion.

Trophoblast cells, derived from the fertilised egg, are in direct contact with the maternal blood by forming a multinucleated syncytia, which facilitates the exchange

of nutrients between the foetal and maternal circulatory systems, while also secreting hormones that maintain pregnancy (Gerbaud & Pidoux 2015). Trophoblast proliferation, migration, and invasion play a crucial role in embryo implantation, maternal circulation, and placental development (Moser & Huppertz 2017). First, trophoblasts invade the endometrium, complete embryo implantation, further invade the decidual and muscular layers of the uterus, and replace the uterine spiral artery endothelial cells, leading to uterine spiral artery recasting. This results in degeneration of the muscular elastic arteries, reduction of intravascular blood flow resistance, and increase of maternal-foetal interface, thus ensuring placental blood supply and foetal development (Zenclussen *et al.* 2014). On the other hand, abnormal growth and differentiation of trophoblast cells cause blockage of erosion, shallow villus implantation, vascular hypoplasia, and inhibition of placental nutrition supply and placental growth, thus leading to pathologic pregnancy, including spontaneous abortion, restriction of foetal growth, and hypertension in pregnancy (Steinberg & Robins 2016, Wong & Cox 2017); however, the underlying mechanism is not yet fully understood.

As a highly conserved essential protein, eukaryotic translation initiation factor 5A (eIF5A) is reported as a stimulant of the initiation phase of protein synthesis, which directly affects about 30–75% of the global translation (Henderson & Hershey 2011). The activity of eIF5A is regulated by hypusination, which is defined as the transfer of 4-aminobutyl moiety of spermidine to the ϵ -amino group of Lys50 (Park *et al.* 2010, Olsen & Connor 2017). eIF5A plays key roles in various cellular processes, including cell cycle, differentiation, migration, and apoptosis, and regulates cellular stress responses via modulation of mRNA transport, protein translation, and elongation (Dias *et al.* 2012, Schuller *et al.* 2017, Turpaev 2018). Studies have demonstrated that eIF5A is involved in the physiological and pathological development of a variety of human diseases, such as human cancers, diabetes, and malaria (Kaiser 2012). In our previous study, we demonstrated that cross-talk between eIF5A and Ca²⁺/calmodulin-dependent protein kinase 1D (CAMK1D) affects HTR8 cells progress (Qin *et al.* 2018). Another study showed that eIF5A was regulated via the plasmacytoma variant translocation 1 (PVT1)/miR-424 axis (Qin *et al.* 2019). However, the role and underlying mechanism of eIF5A in spontaneous abortion remain largely unknown. Here, we sought to elucidate its underlying mechanism. We demonstrated the role of eIF5A in the promotion of HTR8 cells growth. Further research revealed that eIF5A exerted its function via regulation of methyltransferase-like protein 14 (METTL14) expression.

Materials and methods

Cell culture and reagents

The human chorionic trophoblast cell line HTR8 was routinely maintained in our laboratory as recommended by the American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Donghuan Biotech, Shanghai, China) supplemented with 10% foetal bovine serum (FBS; Gibco), 2 mmol/L L-glutamine, 100 µg/mL streptomycin, and 100 units/mL penicillin (Donghuan Biotech) in a humidified atmosphere of 5% CO₂ at 37°C.

RNA interference and plasmid construction

The specific siRNAs against eIF5A and METTL14 were obtained from GenePharma Co., Ltd. (Shanghai, China) and stored at –80°C until further use. The siRNA sequences were as follows: eIF5A-siRNA-1, sense: 5'-UCGAGAUGUCUACUUCGAAdTdT-3'; eIF5A-siRNA-2, sense: 5'-GGCCAUGUAAGA-UCGUCGAdTdT-3'; eIF5A-siRNA-3, sense: 5'-GUCGAGA-UGUCUACUUCGAdTdT-3'; METTL14-siRNA-1, sense: 5'-CAAAGATGAGCAGAGAGAAATTGCT-3'; METTL14-siRNA-2, sense: 5'-CATCAGGCTAAAGGATGAGTTAATA-3'; and METTL14-siRNA-3, sense: 5'-GATCCAAAGGCTGTCTTTCAGAGAA-3'.

The eIF5A and METTL14 were cloned into the pcDNA3.0 plasmids. The cDNA of eIF5A and METTL14 was obtained by whole gene synthesis (Genewis, NJ, USA).

Cell transfections

The siRNAs and plasmids were transfected into cells using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol. In brief, HTR8 cells were cultured according to specific experimental requirements. The next day, the old medium was replaced with opti-MEM (Invitrogen) and the cells were transfected with the siRNAs. After 6 h, opti-MEM was replaced with DMEM containing 10% FBS. After 48 h of incubation, the cells were harvested and used for further experiments.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from the clinical tissue samples and cells using RNA extraction reagent (Donghuan Biotech) according to the manufacturer's protocol and quantified using a spectrophotometer (Tiangen, Beijing, Shanghai). cDNA was synthesised using the SmartQuant™ cDNA synthesis kit with gDNase (Donghuan Biotech) according to the manufacturer's protocol. qRT-PCR was performed on the LightCycler® 96 Real-Time PCR System (Roche) with the Universal SYBR Green qRT-PCR Master Mix (Donghuan Biotech) according to the manufacturer's instructions. Target gene expression was normalised to β -actin and expressed as arbitrary units compared to the control group. The 2^{– $\Delta\Delta C_t$} method was used to analyze the PCR data. The primer sequences used were as follows: eIF5A, forward: 5'-GGACTTCGAGACAGGAGATGC-3' and reverse: 5'-TCATTCCTTTTGATGTTGGGGAC-3'; METTL14, forward: 5'-AATCTGACCGAGGAGGTGGA-3' and reverse: 5'-AGAAA-GTTGGGTA-TCGAGGTG-3'; β -actin, forward: 5'-GTCATTCC-AAATATGAGATGCGT-3' and reverse: 5'-GCTATCACCTC-CCCTGTGTG-3'.

Western blotting

Proteins were isolated from the cells and tissues using RIPA buffer containing 1% PMSF (Sigma-Aldrich) and the protein concentration was measured with a BCA detection kit (Donghuan Biotech). Protein samples were separated on sodium dodecyl sulphate-polyacrylamide gels and transferred onto polyvinylidene difluoride membranes (Millipore). Membranes were then blocked with 5% non-fat milk in phosphate buffered saline (PBS) with Tween 20 (PBST) at room temperature (RT, 25°C) for 1 h and incubated with primary antibodies overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated secondary antibody at RT for 1 h. The proteins were detected by enhanced chemiluminescence (Donghuan Biotech). The following antibodies were used in this study: anti-eIF5A antibody (Abcam; 32443; dilution: 1:2000), anti-METTL14 antibody (Abcam; 220030; dilution: 1:2000), and anti- β -actin antibody (Abcam; 179467; dilution: 1:5000).

CCK-8 assay

Cell viability was assessed using a CCK-8 detection kit (Donghuan Biotech). Briefly, transfected cells were seeded in triplicates in 96-well plates at a concentration of 5×10^3 cells/well. Next day, CCK-8 reagent (10 µL) was added to each well

and incubated at 37°C for 3 h. The optical density at 450 nm was measured using a microplate reader (PerkinElmer).

EdU assay

DNA synthesis in proliferating HTR8 cells was measured with the EdU detection kit (Donghuan Biotech) according to the manufacturer's instructions and as described previously. Briefly, cells were seeded in 48-well plates at a concentration of 5×10^3 cells/well. Next day, EdU (150 μ L) was added to each well and incubated at 37°C for 2 h. The medium was then substituted with 4% paraformaldehyde and incubated at RT for 30 min. After washing with PBS, 0.5% Triton X-100 was added and incubated at RT for 25 min. Next, the cells were stained with Apollo reaction liquid, and the nucleus was stained with Hoechst 33,342 (Solarbio, Beijing, China) at RT for 15 min. Cells were visualized and analysed using a fluorescence microscope (Leica Microsystems).

Cell migration assay

The cell migration potential of HTR8 cells was detected by transwell assay (BD Biosciences). Briefly, transfected cells were seeded on the upper side of the polycarbonate transwell filter in the top chamber at a concentration of 4.5×10^5 cells/well in 1% FBS-containing medium. The lower chamber was filled with 0.6 mL medium containing 10% FBS. Next day, the cells in the upper surface of the chambers were scraped off, and the migrated cells in the lower surface were stained with 0.1% crystal violet at RT for 15 min and air-dried. Cells were analysed under a microscope.

Library preparation and RNA sequencing

Cells transfected with three different siEIF5As or siNC were used for RNA-seq analysis to identify DEGs in both eIF5A knockdown and control cells. Total RNA was isolated from each group of cells using TRIzol reagent. Sequencing libraries were generated using the TruSeq RNA Sample Preparation Kit (Illumina). Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in an Illumina proprietary fragmentation buffer. First-strand cDNA was synthesized during random oligonucleotides and SuperScript II. Second-strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and the enzymes were removed. After adenylation of the 3' ends of the DNA fragments, Illumina PE adapter oligonucleotides were ligated to prepare for hybridization. To select cDNA fragments of the preferred 200 bp in length, the library fragments were purified using the AMPure XP system (Beckman Coulter, Beverly, CA, USA). DNA fragments with ligated adaptor molecules on both ends were selectively enriched using Illumina PCR Primer Cocktail in a 15 cycle PCR reaction. Products were purified (AMPure XP system) and quantified using the Agilent high-sensitivity DNA assay on a Bioanalyzer 2100 system (Agilent). The sequencing library was then sequenced on a HiSeq

platform (Illumina). All Illumina-assembled unigenes were compared with the Ensembl (<http://www.ensembl.org/>), GO database (<http://www.geneontology.org/>), and KEGG database (<http://www.genome.jp/kegg>). The RNASeq was performed by Personalbio Co., Ltd. (Shanghai, China).

Chromatin immunoprecipitation assay (ChIP)

ChIP assay was performed using the ChIP assay kit (Millipore) according to the manufacturer's protocol. Briefly, after HTR8 cells were subjected to different treatments, the medium was discarded. Cells were then washed in ice-cold PBS and cross-linked with 1% formaldehyde. Cross-linking was stopped by the addition of 0.15 M glycine. Next, cells were harvested, and ChIP assay was performed. Enrichment of METTL14 mRNA was measured using qRT-PCR. The input DNA was used as the control.

Clinical human tissue specimens

All patients, including those with completed spontaneous abortions (6–10 weeks gestation, SA group) or elective induced abortions (6–10 weeks gestation, control group), signed the informed consent form. The villi tissues from aborted foetuses were isolated using surgical scissors and immediately stored at -80°C until further use. Samples showing any physical injury, foetal chromosomal abnormalities, or maternal infection were discarded. This study was reviewed and approved by the Research Ethics Committee of International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University.

Statistical analysis

Values are expressed as means $\pm$ s.d. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software). Differences between two groups were analysed by Student's *t*-test. Comparisons between more than two groups were made using one-way ANOVA. *P* values less than 0.05 were considered statistically significant.

Results

Knockdown of eIF5A reduces the viability, proliferation, and migration of HTR8 cells

To investigate the role of eIF5A in HTR8 cells, we first designed and synthesized siRNAs against eIF5A (siEIF5As) and constructed the eIF5A overexpression clone. qRT-PCR and Western blot analyses were then used for validation. Results showed that all the three siEIF5As efficiently inhibited eIF5A expression, while the overexpression clone promoted eIF5A expression in HTR8 cells (Fig. 1A and B).

Next, Cell Counting Kit-8 (CCK-8), 5-ethynyl-2'-deoxyuridine (EdU), and transwell assays were employed to measure the effect of eIF5A on HTR8 cells viability, proliferation, and migration, respectively. The results of CCK-8 assay showed that eIF5A knockdown

Figure 1 Knockdown of eIF5A reduces the viability, proliferation, and migration of HTR8 cells. (A) qRT-PCR and Western blotting to test the validity of the eIF5A overexpression vector in HTR8 cells. (B) qRT-PCR and Western blotting to test the validity and specificity of siRNAs against eIF5A in HTR8 cells. (C) CCK-8 assay to assess HTR8 cells viability after transfection with si-eIF5A, pcDNA-eIF5A, or negative control for 48 h. (D) EdU assay to evaluate HTR8 cells proliferation after transfection with si-eIF5A, pcDNA-eIF5A, or negative control for 48 h. (E) Transwell assay to detect HTR8 cells migration after transfection with si-eIF5A, pcDNA-eIF5A, or negative control for 48 h. β -Actin was used as a control. Each experiment was repeated at least three times. * $P < 0.05$, ** $P < 0.01$.

reduced HTR8 cells viability (Fig. 1C). EdU assay results revealed that eIF5A knockdown inhibited the proliferation of HTR8 cells (Fig. 1D). In addition, the results of transwell assay showed that downregulation of eIF5A decreased the migration potential of HTR8 cells (Fig. 1E). Collectively, these results demonstrated that repression of eIF5A reduces the viability, proliferation, and migration of HTR8 cells.

Gene expression profiles and differentially expressed genes (DEGs) in eIF5A knockdown trophoblast cells

Considering the role of eIF5A in translation initiation, we proposed that eIF5A might exert its function in trophoblast cells through modulation of proteins. To further identify the underlying mechanism of eIF5A, we used RNA-seq to screen the downstream targets of eIF5A in control and eIF5A knockdown HTR8 cells and the data were uploaded in database NCBI (Accession number: PRJNA574732). Transcriptome sequencing was based on the Illumina HiSeq platform using a 150 cycle paired-end sequencing strategy. FastQC was used for quality control. A quality score $Q > 20$ of reads were used for downstream analysis. HTSeq v0.6.1 was used to count the numbers of reads mapped to each gene and gene expression levels were presented as RPKM. We used the DESeq to detect DEGs with the gene symbol annotation (FDR < 0.05 , fold change > 2). The heatmap and volcano plot of global transcriptome changes in siNC-transfected cells and sielF5A-transfected HTR8

cells are shown in Fig. 2A and B. A total of 19,962 RNA transcripts were identified in the cells, of which 19,040 were considered to be stably expressed. We identified 598 upregulated and 740 downregulated mRNAs in HTR8 cells transfected with sielF5A compared to those in siNC-transfected cells. These results demonstrated that eIF5A knockdown dramatically altered the gene expression profile in trophoblast cells.

To validate the RNA-seq based expression of DEGs, qRT-PCR analysis was employed. Using bioinformatics analysis, seven upregulated mRNAs (FAM186B, GATA1, MYOM2, RBAK, SLC10A4, TMEM232, and ZNF804A) and seven downregulated genes (METTL14, ACOX2, MARVELD3, FAM20A, IL12RB1, MAP1LC3B2, and CTD-3105H18.14) were identified. As shown in Fig. 2C, the qRT-PCR results of all up- and downregulated genes (seven out of seven) confirmed of the reliability of the RNA-seq results.

eIF5A regulates METTL14 expression via interaction with the METTL14 promoter in HTR8 cells

To investigate the correlation between METTL14 and eIF5A, HTR8 cells were transfected with sielF5A, pcDNA-eIF5A, and their respective controls. After 48 h, qRT-PCR and Western blotting were performed to measure METTL14 expression. The results showed that eIF5A knockdown inhibited METTL14 expression, while overexpression of eIF5A upregulated METTL14 expression in HTR8 cells (Fig. 3A and B). Taken together,

Figure 2 Gene expression profiles and DEGs in eIF5A knockdown trophoblast cells. (A) Hierarchical clustering of all DEGs based on Z-score normalised FPKM values in three internode segments in HTR8 cells transfected with sielF5A or siRNA negative control for 48 h. (B) The distribution of all genes with respect to significance (y-axis) vs fold changes (x-axis). (C) qRT-PCR analysis to validate RNA-seq based mRNA expression levels. Fourteen differentially expressed mRNAs (DEGs) were validated. The expression of genes was normalised to the expression of β -actin. * $P < 0.05$, ** $P < 0.01$.

these results indicated that eIF5A regulated METTL14 expression in HTR8 cells.

To further explore the mechanism by which eIF5A regulates METTL14 expression, we performed the ChIP assay. The results showed that METTL14 promoter activity was dramatically elevated compared to the IgG control (Fig. 3C). In summary, these observations suggested that eIF5A regulates METTL14 expression via interaction with the METTL14 promoter in HTR8 cells.

Knockdown of METTL14 reduces the viability, proliferation, and migration of HTR8 cells

To examine the role of METTL14 in HTR8 cells, we first designed and synthesised specific siRNAs against METTL14 (siMETTL14s) and constructed the METTL14 overexpression clone. qRT-PCR and Western blotting were then used for validation. The results showed that all three siMETTL14s efficiently inhibited METTL14 expression, while the overexpression clone promoted METTL14 expression in HTR8 cells (Fig. 4A and B).

Next, CCK-8, EdU, and transwell assays were employed to assess the role of METTL14 in HTR8 cells viability, proliferation, and migration. The results of CCK-8 assay showed that METTL14 knockdown reduced HTR8 cells viability (Fig. 4C). EdU assay results revealed that repression of METTL14 inhibited HTR8 cells proliferation (Fig. 4D). In addition, the results of transwell assay showed that downregulation of METTL14 decreased the migration potential of HTR8 cells (Fig. 4E). Collectively, these results demonstrated that inhibition of METTL14 expression reduces the viability, proliferation, and migration of HTR8 cells.

eIF5A exerts its function via regulation of METTL14 expression in HTR8 cells

Our results showed that both eIF5A and METTL14 play a role in the inhibition of various cellular processes in HTR8 cells. Given that METTL14 expression is regulated by eIF5A, we speculated whether modulation of METTL14 could affect the role of eIF5A in HTR8 cells. The results of CCK-8 assay showed that overexpression of METTL14 suppressed siEIF5A-induced reduction of HTR8 cells viability (Fig. 5A). EdU assay results showed that METTL14 overexpression reversed the siEIF5A-induced inhibition of HTR8 cells proliferation (Fig. 5B). In addition, the results of transwell assay showed that overexpression of METTL14 suppressed siEIF5A-induced inhibition of HTR8 cells migration (Fig. 5C). Collectively, these results demonstrated that eIF5A exerts its function in HTR8 cells via regulation of METTL14 expression.

The eIF5A/METTL14 regulatory axis is downregulated in human villi samples of spontaneous abortion

To investigate the function of the eIF5A/METTL14 regulatory axis in spontaneous abortion, qRT-PCR

Figure 3 eIF5A regulates METTL14 expression via interaction with the METTL14 promoter in HTR8 cells. (A) qRT-PCR and Western blotting to detect METTL14 expression in HTR8 cells overexpressing eIF5A. (B) qRT-PCR and Western blotting to detect METTL14 expression in eIF5A knockdown HTR8 cells. (C) ChIP followed by qRT-PCR analysis of the METTL14 promoter in eIF5A knockdown HTR8 cells. β-Actin was used as a control. Each experiment was repeated at least three times. * $P < 0.05$, ** $P < 0.01$.

and Western blotting were performed to measure eIF5A/METTL14 expression in human villi samples of spontaneous and induced abortions. The results showed that the eIF5A/METTL14 regulatory axis was downregulated in spontaneous abortion samples compared with that in induced abortion samples (Fig. 6A and B). In summary, these results revealed that the eIF5A/METTL14 regulatory axis is downregulated in human villi samples of spontaneous abortions.

Discussion

Spontaneous abortion is a disease that seriously impacts on women's physical and mental health. Thus, its underlying mechanism requires urgent investigation. Abnormal trophoblast proliferation, migration, and differentiation

Figure 4 Knockdown of METTL14 reduces the viability, proliferation, and migration of HTR8 cells. (A) qRT-PCR and Western blotting to test the validity of METTL14 overexpression clone in HTR8 cells. (B) qRT-PCR and Western blotting to test the validity and specificity of siRNAs against METTL14 in HTR8 cells. (C) CCK-8 assay to assess HTR8 cells viability after transfection with siMETTL14, pcDNA-METTL14, or negative control for 48 h. (D) EdU assay to evaluate HTR8 cells proliferation after transfection with siMETTL14, pcDNA-METTL14, or negative control for 48 h. (E) Transwell assay to detect HTR8 cells migration after transfection with siMETTL14, pcDNA-METTL14, or negative control for 48 h. β -Actin was used as a control. Each experiment was repeated at least three times. * $P < 0.05$, ** $P < 0.01$.

Figure 5 eIF5A exerts its function in HTR8 cells via regulation of METTL14 expression. (A) CCK-8 assay to detect the viability of HTR8 cells after indicated treatments. The cells were transfected with: siNC, siEIF5A, or pcDNA-METTL14. (B) EdU assay to evaluate the proliferation of HTR8 cells after different treatments. The cells were transfected with: siNC, siEIF5A, or pcDNA-METTL14. (C) Transwell assay to detect the migration of HTR8 cells after different treatments. The cells were transfected with: siNC, siEIF5A, or pcDNA-METTL14. Each experiment was repeated at least three times. * $P < 0.05$, ** $P < 0.01$.

have been reported to be the main cause of spontaneous abortion (Steinberg & Robins 2016, Wong & Cox 2017). Thus, strategies to inhibit abnormal trophoblast function are needed for the treatment of spontaneous abortion. In this study, we aimed to detect the role and mechanism of eIF5A in the growth and migration of trophoblast cells. We first demonstrated that eIF5A induced trophoblast cell viability, proliferation, and migration. Next, we showed that this effect was mediated via upregulation of METTL14 expression. Further, we demonstrated that the eIF5A/METTL14 pathway was downregulated in human villi samples of spontaneous abortion. Hence, our findings suggest that eIF5A modulates trophoblast cell function via regulation of METTL14 expression and that the eIF5A/METTL14 pathway may serve as a potential target in the diagnosis and treatment of spontaneous abortion, but more research is needed.

eIF5A is the only eukaryotic protein known to be hypusinated. Studies have revealed that eIF5A is involved in various cellular processes and human diseases via modulation of mRNA transport, protein translation, and elongation (Dever *et al.* 2014, Pardo *et al.* 2017). In our previous study, we found that PVT1 could regulate miR-424 and miR-424 could modulate eIF5A expression in HTR8 cells. However, limited numbers of studies have systematically screened and validated the downstream target genes of eIF5A. Here, in order to elucidate the exact mechanism of action of eIF5A in HTR8 cells, RNA-seq analysis was performed to screen the downstream target genes of eIF5A. Based on the results, 598 upregulated and 740 downregulated mRNAs were identified in siEIF5A-transfected HTR8 cells. Further, qRT-PCR assay was applied to test the validity and reliability of the RNA-seq results. Collectively, these observations provide new

Figure 6 The eIF5A/METTL14 regulatory axis is downregulated in human villi samples of spontaneous abortion. (A) qRT-PCR analysis of eIF5A expression in human villi samples of spontaneous and induced abortions. (B) qRT-PCR analysis of METTL14 expression in human villi samples of spontaneous and induced abortions. (C) Western blot analysis of eIF5A and METTL14 expression in human villi samples of spontaneous and induced abortions. *n*, no. of samples; SA, spontaneous abortion; Control, elective induced abortion.

insights into the function and underlying mechanism of eIF5A in HTR8 cells. In this study, we found that eIF5A knockdown inhibited both the mRNA and protein levels of METTL14. Further, downregulation of METTL14 inhibited the effects of eIF5A overexpression in HTR8 cells. Hence, METTL14 was identified as a potential downstream target gene of eIF5A.

Our study showed that METTL14 knockdown reduced HTR8 cells viability, proliferation, and migration. Furthermore, overexpression of METTL14 was found to reverse the effects of eIF5A knockdown in HTR8 cells. These results indicated that METTL14, like eIF5A, played a key role in trophoblast function. METTL14, a key component of the methyltransferase complex that consists of METTL14, METTL3, and WTAP, has been reported to modulate N⁶-methyladenosine (m⁶A) modification. Recently, m⁶A modification has gained increasing attention from scientists due to its key roles in various cellular processes, disease physiology, and pathology via regulating gene expression at both transcriptional and translational levels (Yue *et al.* 2015, Patil *et al.* 2018, Tong *et al.* 2018). However, in this study, we did not investigate whether METTL14 exerted its function via modulation of m⁶A. In future studies, we aim to investigate the underlying mechanism of METTL14 in HTR8 cells.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Y L conceived and designed the experiments; X Q, Y C, and J Y performed the experiments; X L and W Z analysed the data and drafted the article, with additional editing by X Q, Y C, and J Y. All authors approved the final article.

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