BMC Pregnancy and Childbirth

RESEARCH

Open Access

Maternal plasma extracellular vesicles tsRNA as potential biomarkers for assessing preterm labor risk

Xinrui Sun[1,2†], Ying Feng[7†], Liang Xie[8†], Yilun Wu[1,2†], Jia Mao[9], Yi Zhang[1,2], Linyu Zhang[1,2], Xin Yuan[1,2], Jiangping Ni[10], Xue Xiao[2,5*], Hanmin Liu[5,6*], Li Dai[3,4,5*] and Fang Ma[1,2*]

Abstract

Background Spontaneous preterm labor (PTL) accounts for approximately 70% of preterm births, posing significant risks to both maternal and neonatal health. Current predictive biomarkers lack sufficient reliability, underscoring the need for non-invasive and dependable indicators. Emerging research indicates that tRNA-derived small RNAs (tsRNAs) are involved in various diseases; however, their potential association with PTL remains underexplored.

Methods Bioinformatics analyses of public GEO datasets (PRJNA415953 and PRJNA428989) were conducted to identify tsRNAs associated with PTL. Validation was performed using plasma extracellular vesicles samples collected at 12 weeks of gestation from PTL patients ($n=45$) and healthy controls ($n=38$). Functional assays were used to assess the impact of tsRNA1 (tRNA-Gly-GCC-5p-tRF-921) on extravillous trophoblast (EVT) function, including apoptosis, migration, invasion, and endothelial-like tube formation in HTR8/SVneo cells. Transcriptomic sequencing was conducted to identify tsRNA1-mediated pathways, and DNA methylation patterns were predicted based on the transcriptomic data. Statistical significance was determined using Student's t-test.

Results Two tsRNAs, tsRNA1 and tsRNA3 (tRNA-Gly-GCC-5p-tR-half-368), were significantly upregulated in PTL patient samples compared to controls. Overexpression of tsRNA1 impaired EVT function, increased apoptosis, and altered DNA methylation profiles, implicating its critical role in PTL mechanisms.

Conclusions This study identifies tsRNA1 as a key regulator of EVT dysfunction and placental pathology in PTL. The findings provide novel insights into the mechanistic role of tsRNAs in PTL and highlight tsRNA1 as a promising biomarker for early risk stratification and prediction of the condition.

†Xinrui Sun, Ying Feng, Liang Xie, and Yilun Wu these authors contributed equally as co-first authors.

*Correspondence:
Xue Xiao
xiaoxue@schxfy.com
Hanmin Liu
liuhm@scu.edu.cn
Li Dai
daili@scu.edu.cn
Fang Ma
mafangmed@126.com

Full list of author information is available at the end of the article

Clinical trial number Not applicable.

Keywords Preterm birth, tsRNAs, Biomarkers, Extravillous trophoblasts, DNA methylation

Introduction

Preterm birth (PTB), defined as delivery before 37 weeks of gestation, is a serious complication of pregnancy that poses significant risks to both maternal and neonatal health. PTB may result from spontaneous preterm labor (PTL, uterine contractions leading to cervical changes), preterm premature rupture of membranes (PPROM), or medically indicated delivery due to maternal/fetal conditions [1, 2]. Early delivery can result in a range of complications, including respiratory distress syndrome, neurodevelopmental impairments, and increased neonatal morbidity and mortality [3–5]. Maternal health can also be negatively impacted, with studies indicating increased risks of postpartum depression, cardiovascular disease, and metabolic syndrome following preterm birth [6–8]. Therefore, effective interventions to mitigate these risks are urgently needed, underscoring the importance of early predictive measures.

The placenta serves as a critical interface between the mother and fetus during pregnancy, essential for fetal development and maternal-fetal health in mammals [9]. Its primary functions are executed by trophoblast cells, which play vital roles in supporting fetal growth and inducing the physiological adaptations required for pregnancy [10]. During embryonic development, the coordinated functions of extravillous trophoblasts (EVTs) in proliferation, migration, and invasion are crucial for ensuring adequate nutrient supply to the embryo and maintaining a healthy pregnancy [11, 12]. Insufficient EVT invasion can lead to severe obstetric complications, such as preeclampsia, intrauterine growth restriction, and preterm birth [13].

Research indicates that the concentration of extracellular vesicles in maternal plasma significantly increases during the first trimester of pregnancy [14]. This rapid accumulation reflects the dynamic biological activities at the maternal-fetal interface, particularly in regulating placental function. Analyzing extracellular vesicles-enriched plasma allows for early assessment of placental function [15]. There is strong evidence supporting the hypothesis that most pregnancy complications originate from early placental dysfunction [16], and extracellular vesicles in maternal plasma may serve as early indicators of these abnormalities. Specifically, extracellular vesicles are thought to play a crucial role in PTL resulting from placental dysfunction.

Extracellular vesicles (EVs) are particles that are released from cells, are delimited by a lipid bilayer, and cannot replicate on their own [17]. They are found in various body fluids, including blood, urine, and saliva [18]. EVs play a key role in intercellular communication under physiological and pathological conditions by delivering specific cargo, such as endogenous microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and tRNA-derived small RNAs (tsRNAs) [19]. tsRNAs are a class of heterogeneous non-coding RNAs generated from tRNA cleavage, typically ranging from 18 to 40 nucleotides in length, overlapping with the fragment size selected for miRNA sequencing. There is a growing consensus that tsRNA fragments are not passive degradation products but actively participate in various physiological and pathological processes [20, 21]. For instance, tsRNAs have been implicated in promoting tumor proliferation and migration in ovarian cancer [22], enhancing tumor growth and recurrence in lung adenocarcinoma [23], and serving as candidate biomarkers in pancreatic cancer [24]. Therefore, systematically identifying and analyzing tsRNAs from small RNA sequencing data holds significant value for uncovering their roles in various diseases.

Despite extensive research, accurate biomarkers and targeted therapies for spontaneous preterm labor remain elusive, primarily due to an incomplete understanding of the underlying molecular mechanisms [25]. Current clinical markers of PTL, such as inflammatory cytokines and metabolic indicators [26, 27], face limitations in predicting PTL or the development of related complications, such as preeclampsia [28]. Furthermore, many interventions are most effective when initiated within the first trimester of pregnancy, underscoring the necessity of early predictive biomarkers [29]. Therefore, identifying novel biomarkers and elucidating the mechanisms driving these adverse obstetric outcomes are of paramount importance [28]. To date, most studies on preterm birth have primarily focused on the role of miRNAs in this condition [30, 31], with little attention paid to the tsRNA data that is inherently present in sequencing datasets. This study aims to address this gap by analyzing tsRNAs from published datasets to investigate whether tsRNAs exhibit significant differential expression between healthy and preterm birth pregnancies, and to evaluate their potential as early pregnancy biomarkers for predicting preterm birth. Our research identified tsRNAs related to preterm birth-associated pathways and validated these findings using early plasma samples from patients within 12 weeks of gestation. Through functional assays and transcriptomic analysis, we report that tsRNA1 (tRNA-Gly-GCC-5p-tRF-921) can impair

EVT function, leading to placental dysfunction and subsequent preterm birth.

Materials and methods

Bioinformatics analysis of tRNA-derived fragments using public small RNA datasets

We downloaded the small RNA sequencing data from two biological projects (PRJNA415953 and PRJNA428989) from NCBI's SRA database. These databases were selected because they provided high-quality, publicly available datasets that included relevant sample types for our study on preterm labor (PTL). Specifically, the PRJNA415953 dataset included whole blood plasma, extracellular vesicles, and extracellular vesicles-depleted plasma from both PTL and control subjects, which allowed us to perform a detailed analysis of extracellular vesicles tsRNAs. Similarly, PRJNA428989 provided data from whole blood plasma and peripheral blood mononuclear cells (PBMCs), offering a comprehensive view of small RNA expression in blood-related tissues from PTL and control patients. The inclusion of these datasets, which cover different sample types from PTL and control groups, was crucial for understanding the differential expression of tRNA-derived fragments in relation to preterm birth.

The original SRA format data was converted to FASTQ format using the fastq-dump tool in sratoolkit v2.9.6. After conversion, the data was processed using the trim_galore tool to remove adapter sequences and low-quality reads, resulting in high-quality clean data. Subsequently, we annotated the clean data using the Unitas tool. Based on the position and sequence length of the sequencing data on tRNA, we classified the tRNA-derived small RNAs (tsRNAs) into several categories: 5' tRNA fragments (5' tRFs), 3' tRNA fragments (3' tRFs), tRF-1, tRNA leaders, and miscellaneous tRFs.

For differential expression analysis, we analyzed each biological project separately to avoid batch effects. In particular, dataset PRJNA415953, which included whole blood plasma, exosomes, and exosome-depleted plasma, was analyzed independently for each sample type to ensure that any differences due to sample type were accounted for individually. Similarly, dataset PRJNA428989 was also analyzed independently for each sample type, including whole blood plasma and peripheral blood mononuclear cells (PBMCs). This approach ensured that differential expression analyses were performed separately for each sample type (plasma, exosomes, and exosome-depleted plasma), which helped to account for any inherent sample-specific differences.

The expression levels of tsRNAs were normalized to Reads Per Million (RPM), and tsRNAs with expression levels below 1 RPM in over 90% of the samples were excluded. Differential expression analysis was performed using average expression levels, followed by the Wilcoxon test with $p \leq 0.05$ and $|\log2 \text{ (Fold Change)}| \geq 1$ as criteria for significance. Target genes of tsRNA1 were predicted using TargetScan and miRanda, as shown in Table 1, Table S 1, and Supplementary file.

Patients and plasma samples

Plasma samples were prospectively collected from a longitudinal cohort of 45 women diagnosed with spontaneous preterm labor(PTL) and 38 term controls at West China Second Hospital of Sichuan University between January 2019 and May 2022. Preterm birth was defined as spontaneous delivery occurring between 20 + 0 and 36 + 6 weeks of gestation, confirmed by first-trimester ultrasound (crown-rump length, CRL), with regular uterine contractions (≥ 4/20 min) and cervical dilation ≥ 4 cm, excluding iatrogenic causes such as placental abruption, preeclampsia, or fetal anomalies.Blood samples were obtained at 12 ± 1 weeks of gestation during routine first-trimester screening. For comparison, plasma samples were also collected from 38 asymptomatic pregnant women who delivered at full term (gestational age ≥ 37 weeks), matched for maternal age (± 2 years), parity, and BMI (± 10%). All plasma samples were processed within 2 h and stored at − 80 °C until analysis. Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of West China Second Hospital (Approval No. k2019069, k2022039). Baseline patient characteristics are presented in Table 2.

Plasma EVs isolation and validation

Invitrogen™ Total Exosome Isolation Kit (from plasma) was used to isolate exosomes from plasma samples. Plasma samples (100 µL) were mixed with 0.5 volumes of PBS and vortexed. Proteinase K (5 µL) was added and incubated at 37 °C for 10 min. Exosome Precipitation Reagent (0.2 volumes) was added, and the mixture was vortexed and incubated at 2–8 °C for 30 min. After centrifugation at 10,000 × g for 5 min at room temperature, the exosome pellet was resuspended in 100 µL PBS. Transmission electron microscopy (TEM) confirmed exosome morphology, and Western blotting was performed to detect CD63 and CD9 (*Supplementary file*).

RT-qPCR

RNA extraction was performed using TRIzol (Ambion, 15596026) following the manufacturer's protocol. cDNA synthesis was carried out using a reverse transcription kit. Primers were designed by Qingke (Table S 2). qPCR was performed using SYBR Green mix (Invitrogen, 11744500) on a Bio-Rad instrument. The amplification protocol was as follows: 95 °C for 2 min,

Table 1 Differential expression of tsRNA in PTL/control samples from PRJNA415953 EVs

Gene	Mean.t/mean.*n*	Pvalue	Up/Down
tRNA-Gly-GCC-5p-tRF-17	2.621862222	0.012837524	up
tRNA-Thr-CGT-3p-CCA-tRF-29	2.361772895	0.033596435	up
tRNA-Gly-GCC-misc-tRF-1281	2.114896236	0.047307182	up
tRNA-Gly-GCC-misc-tRF-1331	2.145330933	0.028065072	up
tRNA-Gly-GCC-misc-tRF-1313	3.224531826	0.005236508	up
tRNA-Gly-GCC-misc-tRF-1319	4.74730169	0.001859853	up
tRNA-Phe-GAA-3p-CCA-tRF-35	2.279255278	0.000275009	up
tRNA-Asp-GTC-3p-CCA-tRF-63	2.154622734	0.010417362	up
tRNA-Asp-GTC-misc-tRF-764	4.678034663	0.009472847	up
tRNA-Asp-GTC-3p-CCA-tRF-100	2.210976431	0.012561139	up
tRNA-Glu-TTC-3p-CCA-tRF-231	2.468217898	0.006634233	up
tRNA-Gly-GCC-misc-tRF-1812	4.732651495	0.001040497	up
tRNA-Gly-GCC-5p-tRF-72	2.178308841	0.033596435	up
tRNA-Asp-GTC-3p-tR-half-321	0.457857735	0.039975504	down
tRNA-Asp-GTC-3p-tR-half-322	2.330701204	0.006634233	up
tRNA-Gly-GCC-5p-tRF-404	2.487536361	0.019230769	up
tRNA-Gly-GCC-5p-tRF-408	2.041636409	0.039975504	up
tRNA-Gly-GCC-5p-tR-half-131	2.657024261	0.012837524	up
tRNA-Gly-GCC-5p-tR-half-134	4.082629144	0.008329648	up
tRNA-Gly-GCC-5p-tRF-489	2.438994171	0.047307182	up
tRNA-Gly-GCC-5p-tRF-490	2.387718373	0.019230769	up
tRNA-Gly-GCC-5p-tRF-834	2.111077774	0.047307182	up
tRNA-Gly-GCC-5p-tRF-835	2.128999984	0.039975504	up
tRNA-Gly-GCC-5p-tRF-877	2.3111179	0.033596435	up
tRNA-Gly-GCC-5p-tRF-903	2.210494366	0.023307704	up
tRNA-Gly-GCC-5p-tRF-908	2.0663671	0.019230769	up
tRNA-Gly-GCC-5p-tRF-918	2.637504059	0.001040497	up
tRNA-Gly-GCC-5p-tRF-921	2.226079025	0.028065072	up
tRNA-Gly-GCC-5p-tRF-924	2.502293515	0.008329648	up
tRNA-Gly-GCC-5p-tR-half-348	2.112162502	0.023307704	up
tRNA-Gly-GCC-5p-tR-half-353	3.370149877	0.015769061	up
tRNA-Gly-GCC-5p-tR-half-360	4.022701139	0.006634233	up
tRNA-Gly-GCC-5p-tR-half-367	4.303746929	0.001859853	up
tRNA-Gly-GCC-5p-tRF-1168	2.216590293	0.028065072	up
tRNA-Gly-GCC-5p-tR-half-471	3.712479854	0.005236508	up
tRNA-Gly-GCC-5p-tR-half-472	4.17961071	0.019230769	up
tRNA-Gly-GCC-misc-tRF-2111	3.79148224	0.028065072	up
tRNA-Lys-CTT-5p-tR-half-72	2.702633208	0.003181029	up
tRNA-Gly-CCC-5p-tR-half-158	2.686318418	0.001859853	up
tRNA-Gly-CCC-5p-tR-half-164	3.229962815	0.010382291	up
tRNA-Gly-TCC-5p-tRF-362	2.844191665	0.000765488	up
tRNA-Asp-GTC-3p-tR-half-545	0.38340373	0.019230769	down
tRNA-Asp-GTC-3p-tR-half-546	2.161907237	0.010222922	up
tRNA-Glu-CTC-misc-tRF-1174	2.137491452	0.015769061	up
tRNA-Glu-CTC-misc-tRF-1427	2.414439504	0.008329648	up
tRNA-Val-AAC-5p-tR-half-58	2.874233979	0.023307704	up
tRNA-Cys-GCA-3p-CCA-tRF-196	2.191321801	0.010382291	up
tRNA-Glu-TTC-5p-tRF-262	2.015449502	0.010382291	up
tRNA-Glu-CTC-5p-tRF-334	2.284719919	0.015769061	up
tRNA-Glu-CTC-5p-tR-half-239	3.422127292	0.008329648	up
tRNA-Glu-CTC-5p-tR-half-245	3.045597888	0.006634233	up
tRNA-Glu-CTC-5p-tR-half-249	2.823858831	0.001859853	up
tRNA-Val-AAC-3p-CCA-tRF-267	2.071906189	0.003181029	up

Table 1 (continued)

Gene	Mean.t/mean.*n*	Pvalue	Up/Down
tRNA-Leu-CAA-3p-CCA-tRF-66	2.636672184	0.001040497	up
tRNA-Gly-TCC-3p-CCA-tRF-172	2.472525939	0.028065072	up
tRNA-Gly-GCC-3p-tRF-253	2.140689406	0.012837524	up
tRNA-Gly-GCC-3p-CCA-tRF-264	3.143227859	0.012837524	up
tRNA-Gly-GCC-3p-CCA-tRF-278	2.719806809	0.033596435	up
tRNA-Arg-TCG-3p-CCA-tRF-385	2.100615704	0.004102451	up
tRNA-Gly-GCC-3p-CCA-tRF-302	4.030159096	0.000189955	up
tRNA-Thr-CGT-3p-CCA-tRF-216	4.064815801	0.001859853	up
tRNA-Gly-TCC-3p-CCA-tRF-199	2.480110397	0.003181029	up
tRNA-Gly-GCC-3p-tRF-315	2.768494715	0.008329648	up
tRNA-Gly-GCC-3p-tRF-316	2.875183487	0.001040497	up
tRNA-Gly-GCC-3p-tRF-317	3.498649338	0.006634233	up
tRNA-Gly-GCC-3p-CCA-tRF-304	3.482653181	0.002446728	up
tRNA-Gly-GCC-3p-CCA-tRF-305	2.755521984	0.00140056	up
tRNA-Gly-CCC-3p-tRF-247	2.167035862	0.000394085	up
tRNA-Gly-CCC-3p-tRF-249	3.131179865	0.002446728	up
tRNA-Gly-CCC-3p-CCA-tRF-255	2.655616621	0.005236508	up
tRNA-Gly-GCC-misc-tRF-3537	3.935277124	0.006634233	up
tRNA-Gly-GCC-misc-tRF-3625	3.11992763	0.003181029	up
tRNA-Gly-GCC-misc-tRF-3856	2.667586945	0.002446728	up
tRNA-Glu-CTC-misc-tRF-2172	2.367735743	0.047307182	up
tRNA-Gly-GCC-misc-tRF-4383	2.088385924	0.041731251	up
tRNA-Arg-ACG-3p-CCA-tRF-93	2.056623963	0.008329648	up
tRNA-Pro-TGG-misc-tRF-1564	2.353572645	0.033596435	up
tRNA-Gly-GCC-misc-tRF-4608	2.245303641	0.041558127	up
tRNA-Gly-CCC-3p-CCA-tRF-319	2.228167768	0.047307182	up

followed by 39 cycles of 95 °C for 15 s and 60 °C for 30 s. GAPDH was used as the internal control for mRNA normalization, while miR-16 was used for tsRNA. Quantitative analysis was performed using the 2^(−ΔΔCT) method.

Western blotting

Extracellular vesicle proteins were extracted from plasma using the residual organic solution post-RNA extraction. After high-speed centrifugation, the protein supernatant was quantified using a BCA kit (Biosharp, BL521A). A total of 20 μg of protein was resolved on a 10% SDS-PAGE gel at 90 V for 30 min and 120 V for 1 h. Proteins were transferred onto nitrocellulose membranes, which were blocked with 5% milk in TBST for 1 h. Primary antibodies were incubated overnight at 4 °C. The following day, secondary antibodies were applied for 1 h at room temperature. Detection was performed using ECL luminescent reagent (Biosharp, BL523B) and visualized with an imaging system. GAPDH served as a loading control. Antibodies used include CD63 (Santa Cruz, I1520, 1:200), CD9 (Santa Cruz, B2120, 1:150) and GAPDH (ABclonal, AC033, 1:10000). HRP-conjugated secondary antibodies were goat anti-rabbit IgG (ABclonal, AS014, 1:1000) and goat anti-mouse IgG (ABclonal, AS003, 1:1000).

Cell culture

Human HTR-8/SVneo cells were cultured in phenol red-free RPMI 1640 medium (Gibco, C22400500BT), supplemented with 10% fetal bovine serum (Gibco, A3160802). No antibiotics were added to the culture medium to avoid potential interference with cellular responses. Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Cell transfection

For tsRNA transfection, a 20 μM stock solution of tsRNA was prepared by dissolving 5 nmol of tsRNA in DEPC-treated water, aliquoted, and stored at − 20 °C to − 80 °C. Transfection was performed when the cells reached 60–70% confluence using Lipofectamine 3000 (Invitrogen). A mixture of Lipofectamine 3000 (5 μL) and serum-free RPMI 1640 (125 μL) was prepared, followed by the addition of tsRNA1 (10 μL). After incubation at room temperature for 5 min, the mixtures were combined and incubated for an additional 15 min. Transfection complexes were added to the cells to reach a final tsRNA1 concentration of 50 nmol/L.

Table 2 Clinical characteristics of the individuals included in this study

	Overall (*n* = 83)	CTRL[a](*n* = 38)	PTL[b] (*n* = 45)	χ^2	*P*
Gestational age (weeks)		38.8 ± 3.1	30.9 ± 2.6	−15.23 (t test)	**< 0.001***
Drug				0.009	0.923
yes	25 (34.72)	13 (18.06)	12 (16.67)		
no	47 (65.28)	25 (34.72)	22 (30.56)		
Pregnancy complications				1.241	0.538
no	76(92.68)	35 (42.68)	41(50.00)		
Gestational diabetes	5 (6.10)	3 (3.66)	2(2.44)		
preeclampsia	1 (1.22)	0 (0.00)	1(1.22)		
delivery				15.02	**<0.0005***
natural labour	72 (86.75)	27 (32.53)	45 (54.22)		
caesarean birth	10 (12.05)	10 (12.05)	0 (0.00)		
forceps delivery	1 (1.20)	1 (1.20)	0 (0.00)		
premature rupture of membranes				14.72	**<0.0001***
yes	30 (38.46)	6 (7.69)	24 (30.77)		
no	48 (61.53)	24 (39.74)	17 (21.79)		
Fetal weight				39.23	**<0.0001***
<2000 g	27 (34.62)	0 (0.00)	27 (34.62)		
>2000 g	51 (65.39)	38 (48.72)	13 (16.67)		
Placenta weight				48.05	**<0.0001***
<500 g	35 (54.05)	2 (2.70)	33 (40.54)		
>500 g	38 (45.95)	33 (51.35)	5 (5.41)		
Amnionitis				13.76	**0.0002***
yes	21 (21.88)	4 (3.13)	17 (18.75)		
no	49 (78.13)	33 (53.13)	16 (25.00)		
First pregnancy				0.050	0.823
yes	40 (42.22)	18 (15.56)	22 (26.67)		
no	40 (57.78)	19 (26.67)	21 (31.11)		
Abortion				2.967	0.227
no	48 (56.47)	20 (23.53)	28(32.94)		
artificial abortion	21 (24.71)	13 (15.29)	8 (9.42)		
spontaneous abortion	16 (18.82)	6 (7.06)	10 (11.76)		
Paternal smoker				0.312	0.576
yes	27 (40.00)	11 (17.78)	16 (22.22)		
no	55 (60.00)	26 (24.44)	29 (35.56)		
Exposure to smoke					
yes	4(7.27)	0(0.00)	4(7.27)		**0.044***
no	51(92.73)	29(52.73)	22(40.00)		
Maternal education				1.864	0.172
≤bachelor	59 (70.45)	25 (29.55)	34 (13.64)		
≥master	20 (29.55)	12 (40.91)	8 (15.91)		
Paternal education				0.601	0.438
≤bachelor	30 (68.18)	15 (34.09)	15 (34.09)		
≥master	14 (31.82)	4 (9.09)	10 (22.73)		
Maternal income				1.253	0.263
<9000	52 (63.64)	22 (25.00)	30 (38.64)		
>9000	27 (36.36)	15 (18.18)	12 (18.18)		
Paternal income				0.612	0.434
<9000	27 (29.55)	11 (11.36)	16 (18.18)		
>9000	52 (70.45)	26 (31.82)	26 (38.64)		
Live with elders				0.152	0.697
yes	43 (47.73)	21 (20.45)	22 (27.27)		
no	36 (52.27)	16 (22.73)	20 (29.55)		
Area of house (m^2)				5.639	**0.018***

Table 2 (continued)

	Overall ($n=83$)	CTRL[a]($n=38$)	PTL[b] ($n=45$)	χ^2	*P*
<100	39 (54.55)	13 (31.82)	26 (22.73)		
>100	40 (45.45)	24 (11.36)	16 (34.09)		
Early pregnancy nocturia					**0.022***
<3	6 (17.14)	6 (17.14)	0 (0.00)		
≥3	29 (82.86)	13 (37.14)	16 (45.71)		

[a]CTRL: Control group (term deliveries, gestational age ≥ 37 weeks)

[b]PTL: Spontaneous preterm labor group (delivery between 20 + 0 and 36 + 6 weeks of gestation, confirmed by first-trimester ultrasound, with regular uterine contractions and cervical dilation ≥ 4 cm, excluding iatrogenic causes)

Adhesion, migration, and invasion assays

For the adhesion assay, Matrigel (BD, 356234) was used to coat 96-well plates, and 2×10^4 cells were added per well and incubated for 4 h. For the migration assay, a scratch was made in the cell monolayer post-transfection using a sterile pipette tip, and fresh medium was added. Images were captured at 24 and 48 h. Invasion assays were conducted using Matrigel-coated chambers with 5×10^4 cells per well. After 24 h, cells were stained with 0.1% crystal violet, photographed, and counted using ImageJ. Control group results were used for normalization.

Endothelial-like tube formation assay

Matrigel-coated 96-well plates were used to assess endothelial-like tube formation. HTR-8/SVneo cells (3×10^4 cells/well) were seeded in the wells containing 50 µL of Matrigel and cultured for 3 h. Tube formation was quantified by counting branch points and measuring total tube length using ImageJ software.

RNA sequencing and analysis

Control and tsRNA1-overexpressing HTR-8/SVneo cells were cultured for 24 h in complete medium. Total RNA was extracted using TRIzol reagent (Invitrogen), and RNA integrity was assessed using an Agilent 4150 TapeStation system. RNA sequencing was performed by APTBIO (Shanghai, China) using the MGISEQ-T7 6G platform (MGI Tech) with paired-end 150 bp (PE150) reads. Raw sequencing reads were normalized as Fragments Per Kilobase of transcript per Million mapped reads (FPKM) to quantify gene expression levels.

Differential expression analysis was conducted using the DESeq2 R package (v1.20.0) with the following criteria: Raw count-based normalization (DESeq2's median-of-ratios method). Significance thresholds:|log2(Fold change)| ≥1, and adjusted p-value < 0.05. Differentially expressed genes (DEGs) meeting these thresholds were subjected to functional enrichment analysis: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses using the `clusterProfiler` R package (v4.0.5). Enrichment significance threshold: p-value < 0.05 (Fisher's exact test).

Statistical analysis

Data were presented as mean ± SEM from at least three independent experiments. Statistical significance was evaluated using an independent-sample t-test, with significance thresholds set at $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Statistical analyses were performed using GraphPad Prism 10.

Results

Distinct Exosomal tsRNA signatures in preterm birth compared to normal term birth

We analyzed the differential expression of tsRNAs between preterm labor and term birth using datasets PRJNA415953 and PRJNA428989 (analysis process shown in Fig. 1A). In PRJNA415953, a total of 93,038, 66,236, and 41,836 unique tRNA fragments were identified from whole blood plasma, exosomes, and exosome-depleted plasma across 67, 22, and 22 samples, respectively. Similarly, dataset PRJNA428989 revealed 63,715 and 57,254 unique tRNA fragments in whole blood plasma and peripheral blood mononuclear cells (PBMCs) from 45 to 43 samples, respectively. Differential expression analysis of PRJNA415953 indicated 32, 77, and 15 tsRNAs in whole blood, exosomes, and exosome-depleted plasma, respectively. Among these, most tsRNAs were upregulated in PTL, except for two downregulated tsRNAs in the exosome plasma (Table 1) and three downregulated tsRNAs in the exosome-depleted plasma (supplementary file). In dataset PRJNA428989, 12 and 72 differentially expressed tsRNAs were identified in whole blood and PBMCs, respectively, and all were upregulated in PTL (supplementary file). Clustering analysis (supplementary file) demonstrated high sample specificity and within-group consistency for the differentially expressed tsRNAs.

We focused on the exosomal tsRNA profile between PTL patients and controls in dataset PRJNA415953, which revealed significant differences in tRNA-derived fragments (tRFs). The volcano plot clearly illustrates distinct tsRNA expression patterns between the two

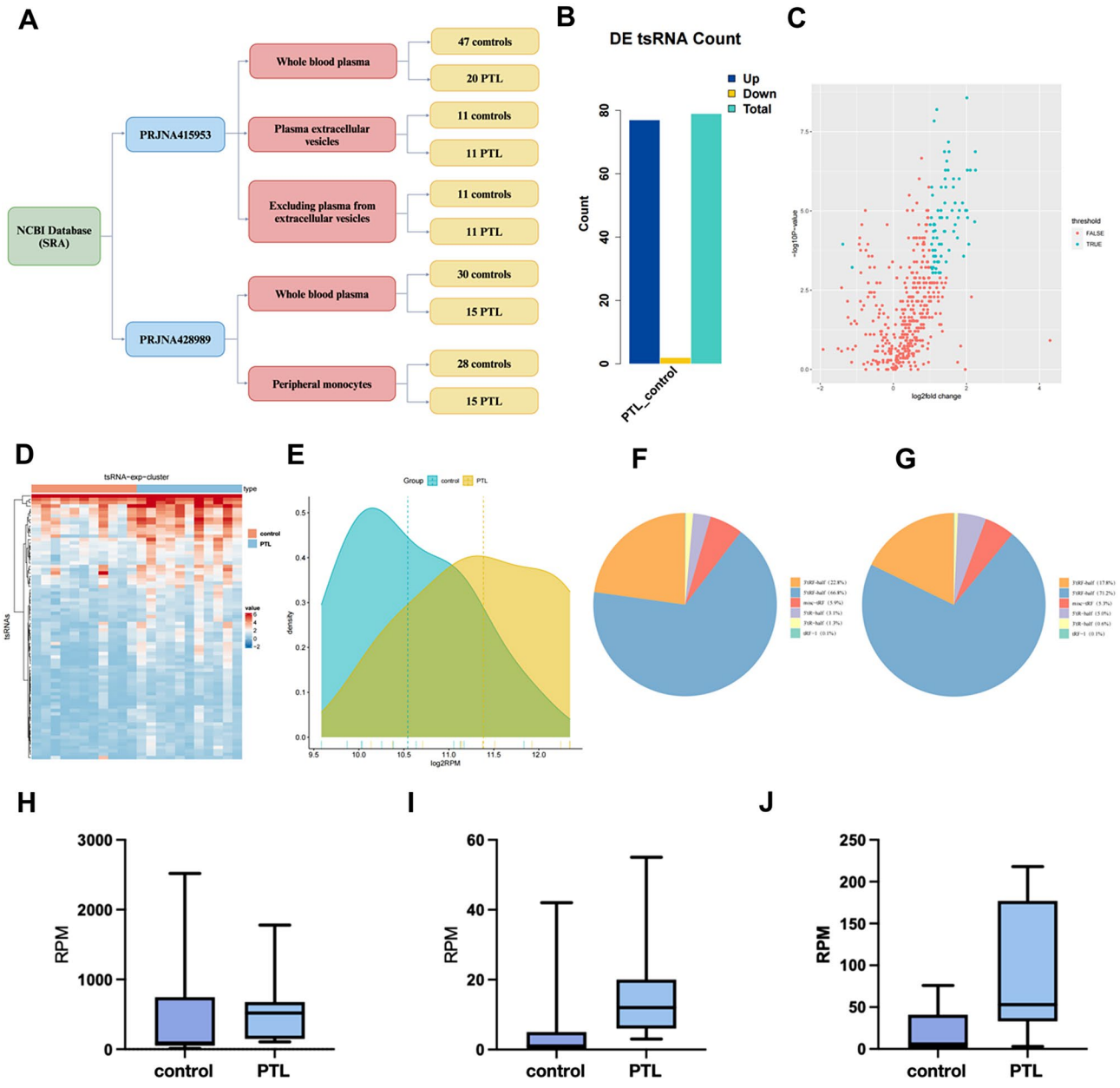

Fig. 1 Identification of tRNA-derived fragments in control and PTL groups. (**A**) Analysis of the tRF processing pathway. (**B**-**D**) Differential expression of tRNA fragments (volcano plot and cluster plot) from the PRJNA415953 dataset in extracellular vesicles (EVs). (**E**) Density distribution of tsRNA abundance in EVs (PRJNA415953). (**F**-**G**) Comparative tsRNA content in EVs from normal and PTL groups across sample types (PRJNA415953). (**H**-**J**) Expression levels of EV-derived tsRNA1 (tRNA Gly GCC-5p-tRF-921), tsRNA2 (tRNA-Asp-GTC-3p-tR-half-547), and tsRNA3 (tRNA-Gly-GCC-5p-tR-half-368) in PTL vs. controls. Note: Partial content was created in BioRender. Wu, Y. (2025) https://BioRender.com/c40q119

groups, and these findings were further confirmed by density distribution and clustering analyses. This suggests that specific exosomal tsRNAs may contribute to adverse pregnancy outcomes, including PTL (Fig. 1B-D). Detailed analyses of other samples from PRJNA415953 and PRJNA428989 are provided in the supplementary file. According to the density distribution of tsRNAs in exosomes from PRJNA415953 (Fig. 1E), tsRNA expression was significantly higher in PTL samples compared to term birth. Analysis of the distribution of different tsRNA types showed that 5'tRF-half fragments predominated in both term birth (Fig. 1F) and PTL exosomes (Fig. 1G).

Research indicates that tsRNAs may function similarly to miRNAs by binding to target mRNAs via the seed sequence at positions 2–7 at the 5' end, thereby inhibiting mRNA expression [32]. We used Miranda and TargetScan software to predict the target genes of these differentially expressed tsRNAs. Genes supported by both tools were

considered definitive targets, and GO and KEGG enrichment analyses were performed on the predicted targets (results for datasets PRJNA415953 and PRJNA428989 are available in the *supplementary file*). From the exosomal samples in PRJNA415953, we identified the following key tsRNAs: tRNA-Gly-GCC-5p-tRF-921, tRNA-Asp-GTC-3p-tR-half-547, and tRNA-Gly-GCC-5p-tR-half-368. In PRJNA428989, key tsRNAs identified in plasma samples included tRNA-Gly-GCC-5p-tR-half-946, tRNA-Val-AAC-5p-tR-half-149, tRNA-Glu-TTC-5p-tR-half-254, and tRNA-Glu-CTC-5p-tR-half-469. These tsRNAs were significantly upregulated in PTL compared to control samples, with exosome tsRNA1 showing particularly high expression compared to tsRNA2 and tsRNA3 (Fig. 1H-J). To streamline the nomenclature, we refer to them as plasma tsRNA1, plasma tsRNA2, plasma tsRNA3, plasma tsRNA4, and EVs tsRNA1, EVs tsRNA2, and EVs tsRNA3 in subsequent sections.

tsRNA1 expression is significantly up-regulated in plasma EVs in preterm labor patients

In our preliminary studies, we analyzed plasma tsRNAs in 25 first-trimester PTL and 25 term birth samples, finding no significant differences in plasma tsRNA expression. However, we observed significant differences in exosomal tsRNA1 and tsRNA3 between PTL and control groups (supplementary file). Therefore, we expanded our sample size in subsequent analyses, focusing exclusively on exosomal tsRNAs from early pregnancy plasma samples to validate the expression levels of EVs tsRNA1, EVs tsRNA2, and EVs tsRNA3 in PTL and term birth cases.

As illustrated in Fig. 2A, plasma samples were collected from 45 PTL patients and 38 gestational age-matched controls. EVs were isolated from maternal plasma using Invitrogen™ Total Exosome Isolation Kit (from plasma), followed by total RNA extraction for downstream analysis. The detailed statistical analysis of the clinical data is provided in Table 2.

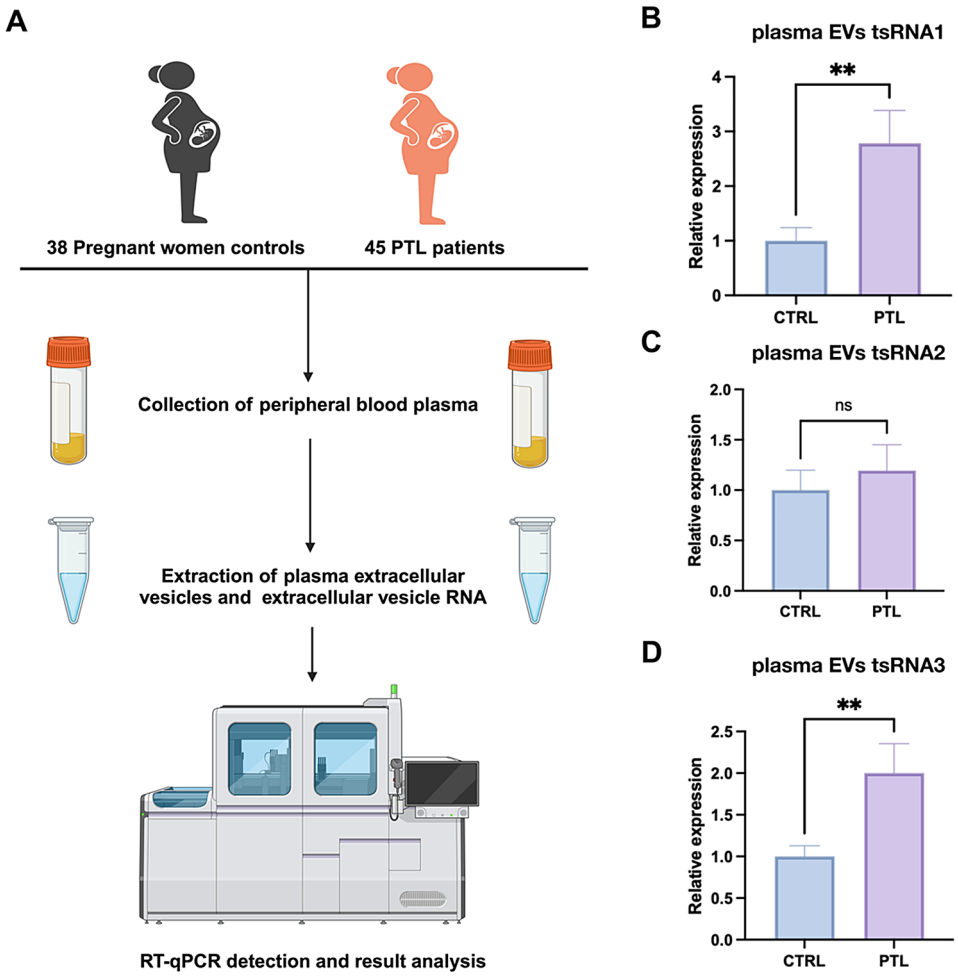

Fig. 2 Elevated levels of tsRNA1 and tsRNA3 in maternal plasma extracellular vesicles. (**A**) Study design: Cohort includes 38 controls and 45 PTL patients with plasma collected for EV analysis. (**B**) tsRNA1 expression in PTL vs. controls. (**C**) tsRNA2 expression (no significant difference). (**D**) tsRNA3 expression in PTL vs. controls. Data: *$P < 0.05$, **$P < 0.01$. Note: Partial content created in BioRender. Wu, Y. (2025) https://BioRender.com/q98a177

To validate the differentially expressed tsRNAs identified in sequencing analysis, RT-qPCR was conducted on selected tsRNAs. The results confirmed that both exosomal tsRNA1 and tsRNA3 were significantly upregulated in PTL patients compared to controls (Fig. 2B and D). Conversely, plasma exosomal tsRNA2 levels did not show a statistically significant difference between the PTL and control groups, suggesting that tsRNA2 may not play a role in PTL pathology (Fig. 2C). These validation results highlight the potential of tsRNAs, especially tsRNA1 and tsRNA3, as biomarkers for predicting preterm birth. The consistent upregulation of these tsRNAs across different methodologies underscores their robustness and potential utility as early diagnostic markers.

In our study, we observed that combining tsRNA1 and tsRNA3 yielded an area under the curve (AUC) of 0.61 (95% CI: 0.46–0.76), indicating modest diagnostic performance. However, integrating these tsRNAs with clinical parameters, such as exposure to smoke, area of house, and early pregnancy nocturia, improved the AUC to 0.83 (95% CI: 0.68–0.99), suggesting that a multi-marker panel may enhance predictive accuracy for preterm birth (Table S 3). Given our sample size, these findings are preliminary. To further investigate the role of tsRNA, we conducted functional assays using the HTR8/SVneo cell line as follows.

tsRNA1 promotes apoptosis without affecting proliferation in EVT cells

To investigate the functional role of tsRNA1 in pregnancy-related complications, we first examined its expression across various EVT cell lines, including BeWo, JEG-3, HTR8/SVneo, and primary EVT cells, using RT-qPCR. The results demonstrated that tsRNA1 expression was significantly higher in the choriocarcinoma-derived BeWo and JEG-3 cell lines compared to HTR8/SVneo and primary EVT cells. This observation is consistent with the cancerous nature of BeWo and JEG-3, which are known to exhibit altered gene expression profiles distinct from normal trophoblast cells [33]. In contrast, HTR8/SVneo cells, which are more physiologically representative of primary EVT cells, exhibited lower levels of tsRNA1 expression [34, 35]. Given that HTR8/SVneo cells more closely mimic in vivo EVT behavior [36], they were selected for subsequent functional analyses (Fig. 3A).

Furthermore, it is important to highlight that HTR8/SVneo cells are capable of secreting EVs, as confirmed by transmission electron microscopy (TEM) images, which are available in the supplementary file. This ability supports their relevance in studying cell-to-cell communication, particularly through exosomal cargo, such as tsRNAs. In addition to tsRNA1, we also evaluated the expression and functional impact of tsRNA3. Despite successful overexpression of tsRNA3 in HTR8/SVneo cells, no significant effects were observed on cell adhesion, migration, or invasion, in contrast to the pronounced effects observed with tsRNA1. The detailed data and supplementary figures regarding tsRNA3's functional impact are provided in the supplementary file for further reference.

Since EVT cell proliferation and apoptosis are critical processes for maintaining normal placental function—ensuring the delivery of nutrients and oxygen to the fetus while also playing a pivotal role in immune regulation at the maternal-fetal interface—proper regulation of these processes is essential [37, 38]. EVT proliferation supports placental growth, while apoptosis facilitates tissue turnover and maintains cell homeostasis, preserving placental integrity and function [39]. Dysregulation of these mechanisms can lead to placental dysfunction and adverse pregnancy outcomes, including fetal growth restriction, preeclampsia, and preterm birth [40]. We next investigated the impact of tsRNA1 overexpression on EVT cell proliferation and apoptosis using HTR8/SVneo cells, which model first-trimester trophoblast behavior. Importantly, our study design aligns with the prospective collection of plasma samples at 12 weeks of gestation, followed by longitudinal tracking of pregnancy outcomes. This approach ensures that our findings are biologically relevant to early placental development and its potential dysregulation in later complications. Results from the CCK8 assay revealed that tsRNA1 overexpression did not significantly affect HTR8/SVneo cell proliferation (Fig. 3B). However, flow cytometry analysis showed a marked increase in both early and late apoptosis in tsRNA1-overexpressing cells, indicating that tsRNA1 promotes apoptosis in EVT cells (Fig. 3C). While apoptosis is a physiological process in early placental remodeling, excessive apoptosis during this critical window may disrupt trophoblast homeostasis [34, 41]. Our findings suggest that tsRNA1-induced apoptosis under pathological conditions could impair EVT function, potentially contributing to complications such as preterm birth.

tsRNA1 inhibits adhesion, migration, invasion, and angiogenesis in EVT cells

The capacity of EVTs in adhesion, migration, invasion, and vascular remodeling are essential for successful placental development and function. Proper EVT cells adhesion to the extracellular matrix, as well as their migratory and invasive capabilities, are key processes for establishing and maintaining placental architecture and ensuring maternal-fetal nutrient exchange. Additionally, EVT cells contribute to spiral artery remodeling, a process crucial for providing sufficient blood flow to the developing fetus. Dysregulation of these processes is often associated with pregnancy complications, including preterm birth,

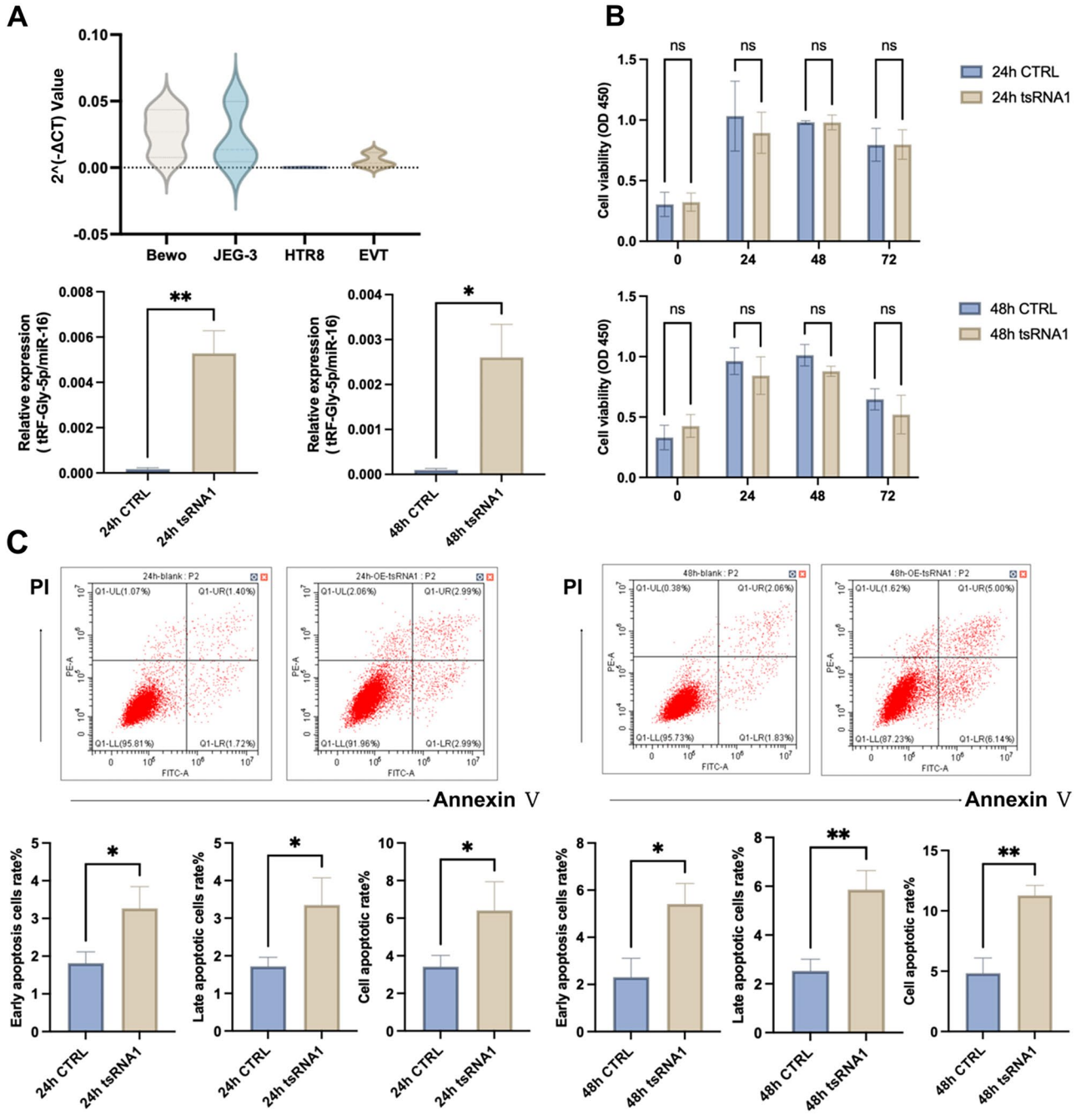

Fig. 3 Effects of tsRNA1 upregulation on cell growth. (**A**) RT-qPCR validation of tsRNA1 expression in Bewo, JEG-3, HTR-8/SVneo, primary EVT Cells and successful overexpression of tsRNA1 in HTR-8/SVneo cells. (**B**) Proliferation assay in HTR-8/SVneo cell (no significant change). (**C**) Apoptosis assay (early, late and total apoptosis). Data: Mean ± SEM of ≥ 3 experiments; *$P < 0.05$, **$P < 0.01$

preeclampsia, and fetal growth restriction, as impaired EVT cells function can lead to inadequate placental development and vascularization. Preterm birth, in particular, has been linked to failures in EVT cells invasion and proper vascular remodeling.

Further experiments demonstrated that tsRNA1 overexpression significantly impaired the adhesive, migratory, and invasive capabilities of HTR8/SVneo cells. Cell adhesion assays revealed a marked reduction in the ability of tsRNA1-overexpressing cells to adhere to the extracellular matrix (Fig. 4A). Additionally, scratch wound healing assays showed that tsRNA1 overexpression significantly reduced the migration potential of HTR8/SVneo cells (Fig. 4B). Furthermore, invasion assays indicated a significant reduction in the invasive capability of tsRNA1-overexpressing cells (Fig. 4C). These results

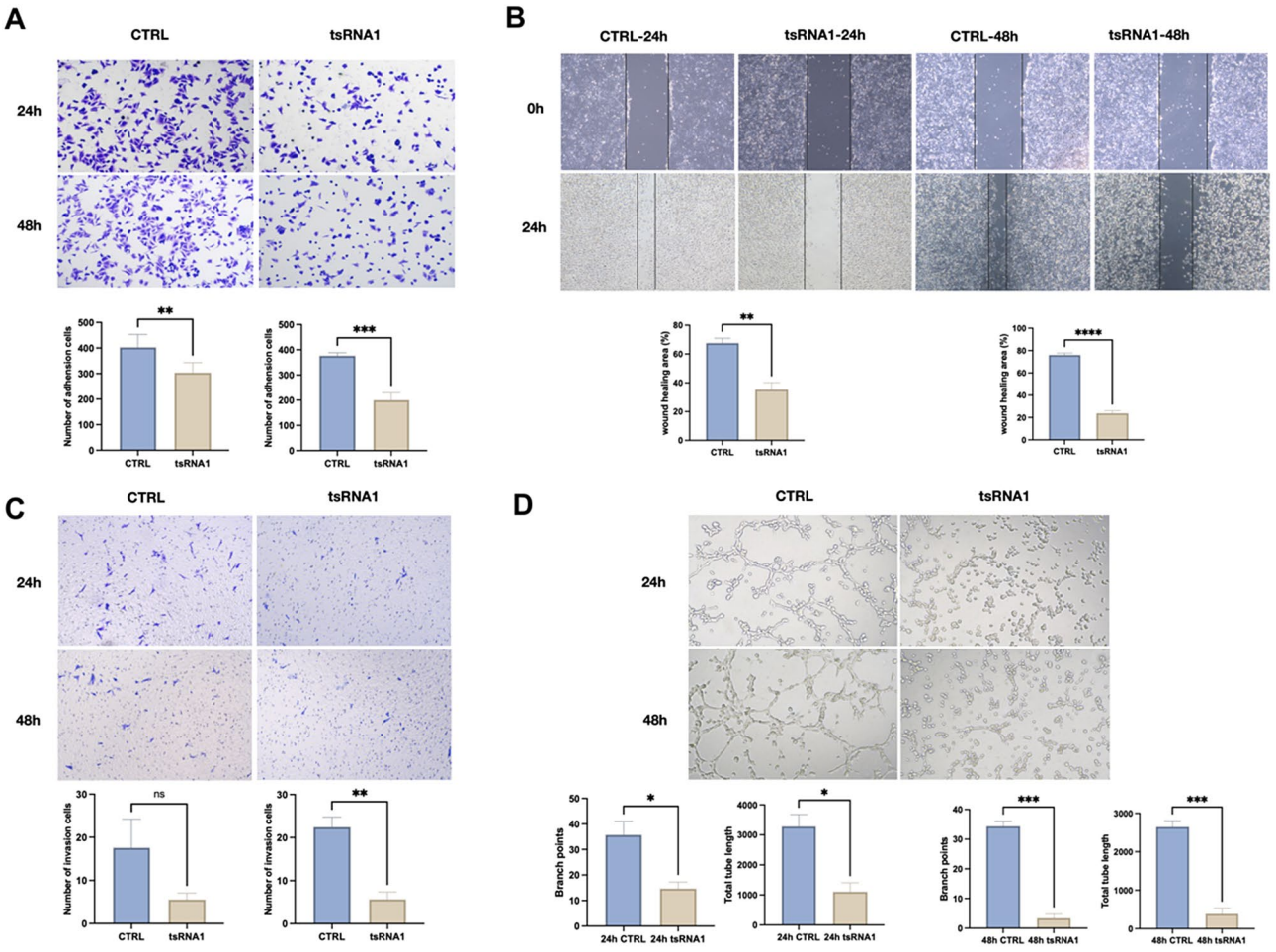

Fig. 4 tsRNA1 Reduces Adhesion, migration, invasion, and endothelial-like tube formation of HTR-8/SVneo cells. (**A**-**C**) Adhesion, migration, and invasion assays after tsRNA1 overexpression. (**D**) Endothelial-like tube formation assay. Data: Mean ± SEM of ≥ 3 experiments; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

suggest that tsRNA1 negatively impacts EVT cells function, potentially contributing to pregnancy complications such as preterm birth by impairing processes critical to placental development. This was further supported by the endothelial-like tube formation assay, where tsRNA1 overexpression resulted in decreased tubular network formation, indicating impaired angiogenesis (Fig. 4D). These findings suggest that tsRNA1 may contribute to adverse pregnancy outcomes by disrupting EVT cells function, particularly in processes essential for placental development and angiogenesis.

Transcriptome and epigenetic analysis

Similar to miRNAs, tsRNAs can bind directly to mRNAs, resulting in mRNA degradation or translational inhibition (Fig. 5G). This mechanism suggests that tsRNA1 may exert post-transcriptional regulatory effects on its target genes.

Transcriptome analysis of tsRNA1-overexpressing HTR8/SVneo cells revealed significant changes in gene expression profiles, with a clear clustering of differentially expressed genes (DEGs) (Fig. 5C). The volcano plot (Fig. 5B) highlighted several genes that were significantly upregulated or downregulated in response to tsRNA1 overexpression, with a threshold of fold change ≥ 2 and FDR < 0.05. Among these DEGs, we focused on ten-eleven translocation 1 (TET1) and DNA methyltransferase 1 (DNMT1) due to their critical roles in DNA methylation regulation. TET1 is involved in DNA demethylation, while DNMT1, a key DNA methyltransferase, facilitates the maintenance of DNA methylation patterns.

We employed bioinformatics tools to predict potential target genes of tsRNA1 and subsequently intersected these predictions with the DEGs from our transcriptome analysis (Fig. 5F, supplementary file). Notably, TET1 was found to be significantly downregulated following tsRNA1 overexpression, indicating a potential post-transcriptional regulatory effect of tsRNA1 on TET1 expression. This prediction was

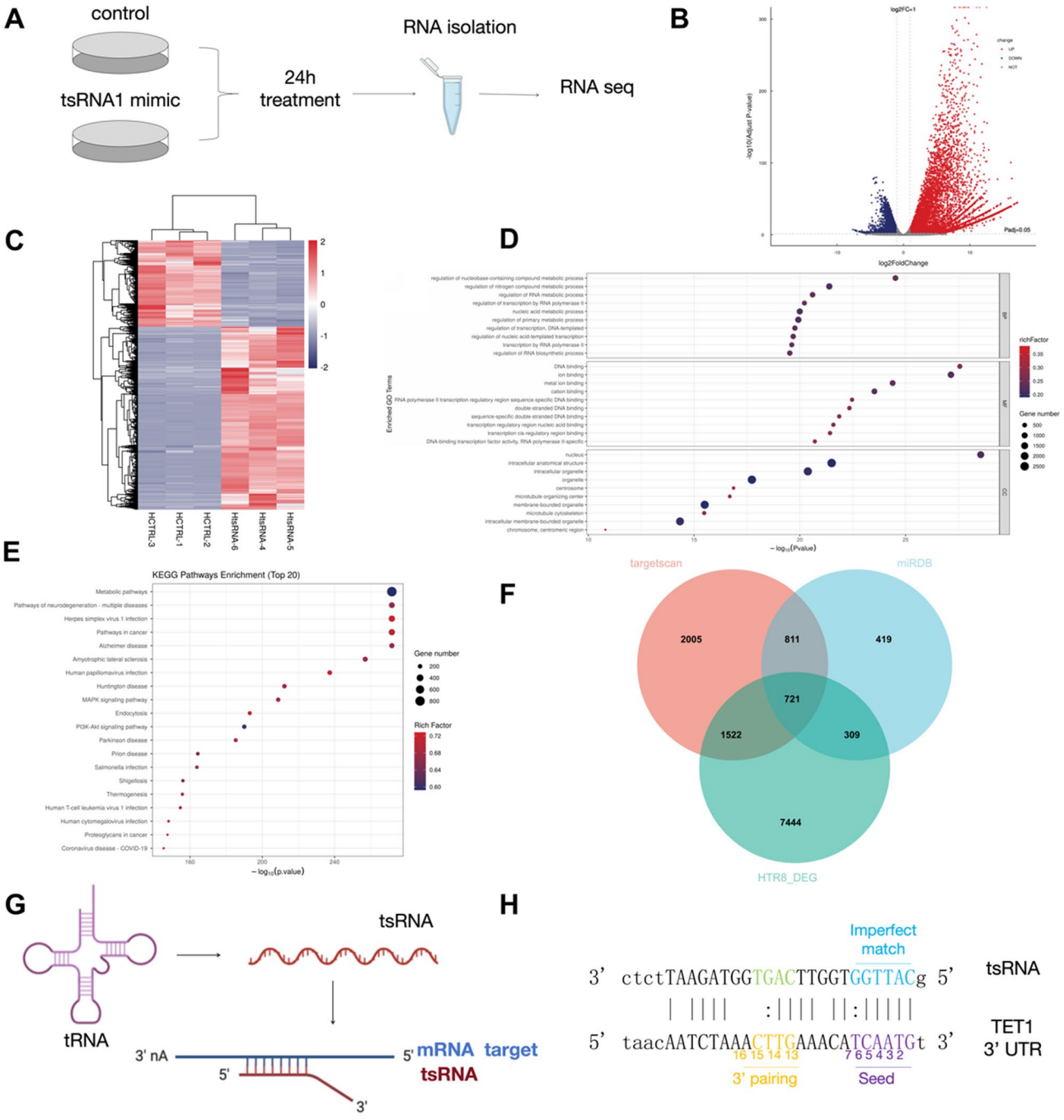

Fig. 5 Transcriptome analysis and tsRNA1 target gene prediction. (**A**) RNA-seq workflow. (**B**) Volcano plot of DEGs (fold change ≥ 2, FDR < 0.05). (**C**) Heatmap of DEG clustering. (**D**) GO analysis. (**E**) KEGG pathway enrichment. (**F**) Venn diagram of target gene overlap. (**G**) Proposed tsRNA1-mRNA interaction. (**H**) Predicted TET1 3'UTR binding. Note: Partial content created with BioRender (Wu, Y. (2024); BioRender.com/z82w555)

confirmed by the observation that tsRNA1 binds to the 3' UTR of TET1 mRNA, potentially leading to its degradation or inhibition of translation (Fig. 5H). While DNMT1 was upregulated, indicating a potential regulatory network where tsRNA1 influences both TET1 and DNMT1. The upregulation of DNMT1 may be a compensatory response to the decreased activity of TET1, as reduced TET1 levels can lead to increased DNA methylation. This interplay suggests that tsRNA1 may promote a hypermethylated state, affecting EVT cells function.

Further validation experiments revealed that tsRNA1 overexpression in HTR8/SVneo cells significantly increased DNMT1 mRNA levels, while suppressing TET1 expression (Fig. 6B-D). Given that DNMT1 is the primary enzyme responsible for maintaining DNA

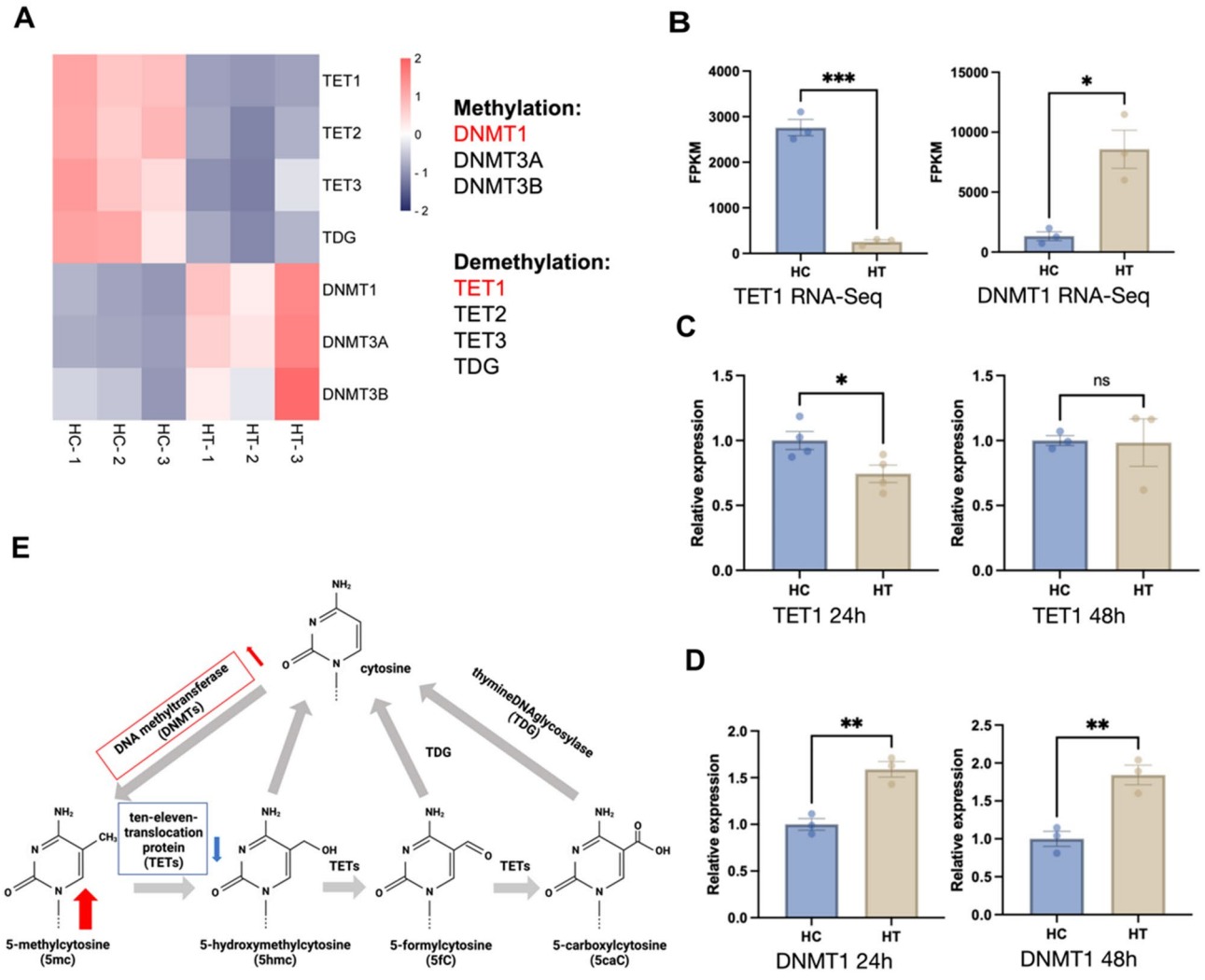

Fig. 6 tsRNA1 affects HTR-8/SVneo cell function by increasing DNA methylation. (**A**) Heatmap of differentially methylated genes. (**B**) TET1 and DNMT1 mRNA changes (transcriptome data). (**C**-**D**) RT-qPCR validation of TET1/DNMT1. (**E**) Model of tsRNA1-induced hypermethylation. Note: Partial content created in BioRender. Wu, Y. (2025) https://BioRender.com/o00f307

methylation patterns, and TET1 catalyzes the oxidation of 5mC to initiate demethylation [42, 43], the observed inverse regulation of these genes suggests a shift toward hypermethylation. This hypothesis is further supported by elevated global 5mC levels in tsRNA1-overexpressing cells (Fig. 6E). While direct measurement of DNMT1 activity or methyl donor metabolites (e.g., SAM) was not performed, our findings align with established models of methylation dysregulation in placental dysfunction [44–46], implicating tsRNA1 as a potential epigenetic modulator in preterm birth pathogenesis.

Discussion

Principal findings

This study identifies maternal plasma EVs-derived tsRNA1 and tsRNA3 as potential biomarkers for predicting the risk of preterm labour (PTL) in pregnant women to reduce or prevent preterm birth (PTB). Our analysis shows significant upregulation of tsRNA1 and tsRNA3 in the plasma EVs of patients with PTL. Notably, tsRNA1 is associated with impaired extravillous trophoblast (EVT) function and increased apoptosis, emphasizing its role in PTL pathogenesis, as shown in Fig. 7.

Results in the context of what is known

Placental dysfunction is closely associated with the pathogenesis of PTB. Currently, the prediction of PTB primarily relies on transvaginal ultrasound (TVUE) measurements of cervical length (CL) [47]. Furthermore, existing research has predominantly focused on predicting PTB through metabolomics analysis and inflammatory markers [48, 49]. However, these methods exhibit

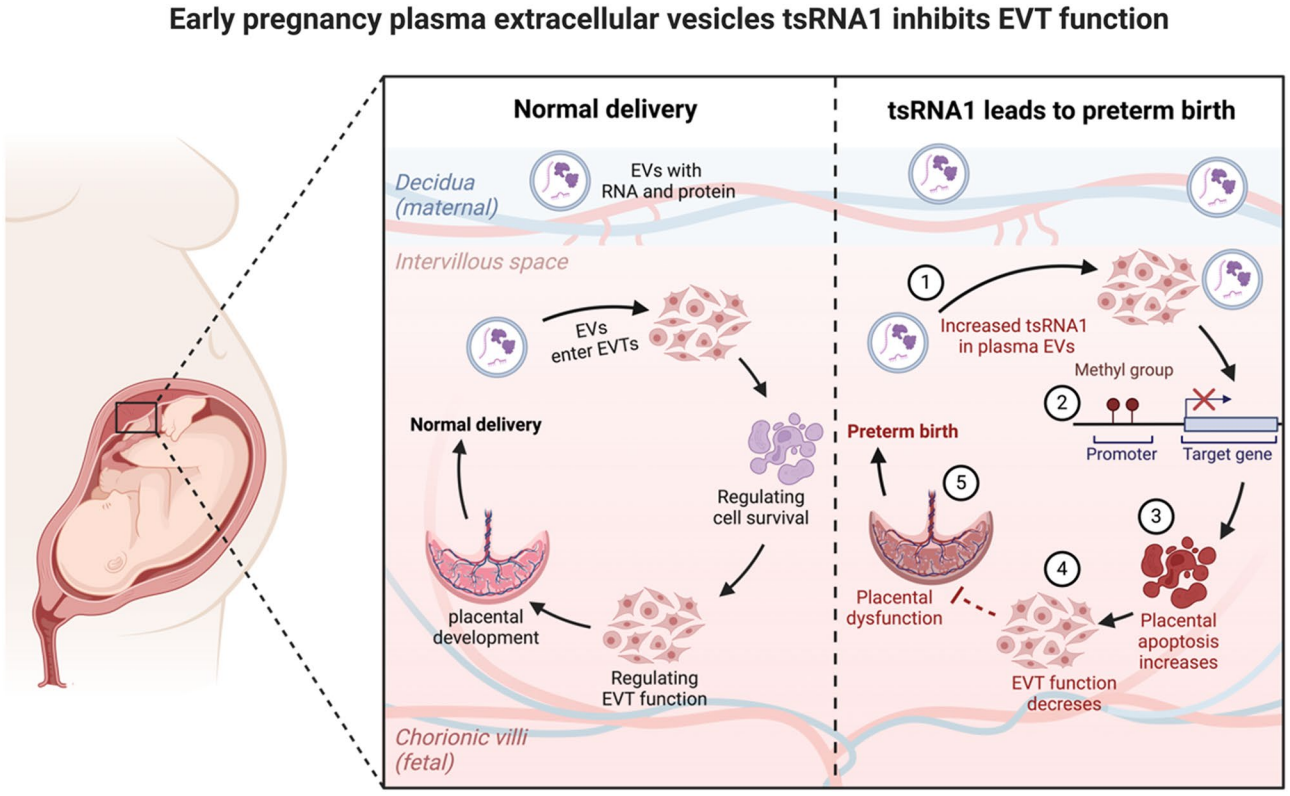

Fig. 7 Proposed mechanism of tsRNA1-Mediated EVT dysfunction in preterm birth. Created in BioRender. Wu, Y. (2025) https://BioRender.com/e93w090

Table 3 Gestational dynamics of tsRNA1 and tsRNA3: hypotheses and mechanisms

Gestational Stage	tsRNA	Expression Trend	Proposed Mechanism
First Trimester	tsRNA1	Significantly elevated	Regulation of epigenetic reprogramming during trophoblast differentiation (DNMT/TET balance) [57–59]
	tsRNA3	Significantly elevated	Early placental development and immune adaptation at the maternal-fetal interface [60, 61]
Second Trimester	tsRNA1	Plateau	Reduced demand for epigenetic remodeling after completion of placental invasion [62, 63]
	tsRNA3	Further elevation	Immune modulation triggered by oxidative stress (e.g., mitochondrial load) or pathogen-associated molecules [64, 65]
Third Trimester	tsRNA1	Late-term elevation	Placental aging or endoplasmic reticulum stress-induced release [66, 67]
	tsRNA3	Pre-labor elevation	Activation of inflammasome pathways (e.g., NLRP3/IL-1β) to initiate labor-associated inflammation [67, 68]

significant limitations, including interindividual heterogeneity, inadequate specificity, and the dynamic fluctuations of biomarkers [50–54]. In contrast, tsRNAs found in plasma extracellular vesicles demonstrate considerable potential as novel early biomarkers due to their stability in body fluids, high specificity, and close association with pathological processes [55, 56]. In this study, we are the first to identify and validate maternal plasma extracellular vesicles tsRNAs as potential biomarkers for predicting the risk of PTL. Our findings reveal a significant upregulation of tsRNA1 and tsRNA3 in the plasma extracellular vesicles of patients experiencing PTL. Notably, tsRNA1 is closely linked to impaired EVT cell function, increased apoptosis, and epigenetic modifications, thereby highlighting its potential role in the pathogenesis of PTL. Overall, tsRNA1 in the early pregnancy plasma extracellular vesicles may serve as a crucial regulatory factor for placental function. Targeting the signaling pathways mediated by tsRNA1 holds promise as an effective strategy for preventing and treating PTL.

The hypothesized gestational dynamics of tsRNA1 and tsRNA3, along with their potential mechanisms, are summarized in Table 3.

In line with these proposed dynamics, we further hypothesize that a pronounced increase in tsRNA1 (≥ 75th percentile of controls) may reflect pathological placental stress. This stress could be driven by factors such as oxidative damage (e.g., from smoke exposure),

failed spiral artery remodeling (linked to uterine resistance), and epigenetic dysregulation, including TET1 suppression and DNMT1 activation (Figs. 5 and 6). In contrast, a modest elevation of tsRNA1 may occur as part of normal physiological adaptation during early placental development, potentially supporting maternal-fetal immune tolerance or metabolic adjustments.

tsRNAs are a novel class of non-coding small RNAs derived from precursor or mature tRNAs and play significant roles in the development and progression of various human diseases [69–71]. Despite this, the specific functions and mechanisms of tsRNAs in PTL remain poorly understood. In our study, we conducted a bioinformatics analysis of sequencing data from the GEO database, specifically the PRJNA415953 and PRJNA428989 datasets, to screen for tsRNAs associated with PTL. Interestingly, we found that most tsRNAs were significantly upregulated in the plasma extracellular vesicles of patients with PTL. Based on their sequence characteristics, tsRNAs can be further categorized into three subclasses: 5' tRNA-derived fragments (5' tRF), 3' tRNA-derived fragments (3' tRF), and stress-induced tRNAs (tiRNAs) [72, 73]. Among these, tsRNA1 (tRNA-Gly-GCC-5p-tRF-921) is classified as a 5' tRF, generated from the 5' end of tRNA cleavage. Utilizing the HTR8/SVneo cell line, we found that overexpression of tsRNA1 significantly increases apoptosis in EVT cells and inhibits their adhesion, migration, and formation of vascular-like structures. This dysfunction in EVT leads to compromised placental function, thereby elevating the risk of PTL [38, 74, 75]. In our previous research, we also identified another tsRNA, tsRNA3 (tRNA-Gly-GCC-5p-tRF-924), which was significantly elevated in the early plasma EVs of pregnant women at risk for PTL. However, overexpression of tsRNA3 did not affect EVT survival or invasiveness, suggesting that tsRNA3 may influence the function of other cell types. We propose two hypotheses for its importance. Firstly, tsRNA3 may modulate decidual macrophages or natural killer (NK) cells to balance pro- and anti-inflammatory signals at the maternal-fetal interface [76]. Secondly, as extracellular vesicle cargo, tsRNA3 might mediate crosstalk between EVT cells and decidual stromal cells, similar to exosomal miRNAs in placental development [77, 78]. Mechanistically, overexpression of tsRNA1 promotes DNA hypermethylation, resulting in persistent epigenetic changes that impair EVT cells function and increase the risk of PTL. Collectively, these findings suggest that signaling pathways mediated by tsRNAs may represent a promising approach for the prediction and intervention of PTL.

Utilizing transcriptomic and bioinformatics analyses, we explored the mechanism by which tsRNA1 inhibits EVT function. Previous studies have shown that tsRNAs are associated with neurodegenerative diseases, metabolic disorders, and cancer [79–81]. Recent findings indicate that tsRNAs regulate the expression of key genes associated with these diseases through various mechanisms, including epigenetic modulation [71, 82, 83]. Specifically, tsRNAs can function through gene silencing, translational reprogramming, and competitive binding with essential proteins [69, 84]. Research has established that DNA methylation is critical for early trophoblast development [85], with the overexpression of DNA methylation-related genes (DNMTs) suppressing trophoblast cell fusion [86]. In contrast, trophoblast cells rely on TET1 binding to prevent the accumulation of repressive DNA methylation [87]. Our transcriptomic analysis revealed that following tsRNA1 overexpression, there is upregulation of differential genes associated with DNMTs (DNMT1, DNMT3A, DNMT3B) and downregulation of TETs (TET1, TET2, TET3, TDG). This change is associated with the accumulation of DNA methylation, potentially leading to impaired EVT function. Furthermore, given that tsRNAs have gene silencing capabilities akin to miRNAs, we hypothesize that tsRNA1 may bind to the 3' UTR of the TET1 gene, further corroborating its role in regulating DNA methylation. The specific mechanisms by which tsRNA1 modulates EVT cells function and its implications for PTL risk warrant further investigation to enhance our understanding of its significance during pregnancy.

Clinical implications

The identification of tsRNA1 and tsRNA3 as biomarkers for PTL risk provides a novel tool for early risk stratification and prediction. Understanding the mechanisms by which tsRNA1 impairs EVT function through DNA hypermethylation could inform clinical strategies to mitigate PTL risk. Further research is needed to validate these findings in larger clinical cohorts and assess the practical applications of tsRNA analysis in routine prenatal care.

Our data indicate that a marked increase in tsRNA1 levels in maternal plasma extracellular vesicles may be associated with placental stress and subclinical dysfunction [88, 89]. In pathological conditions, elevated tsRNA1 might reflect underlying mechanisms such as oxidative stress–induced mitochondrial dysfunction, abnormal vascular remodeling, and dysregulated epigenetic modifications that compromise trophoblast differentiation and placental barrier integrity [88–90]. Conversely, it is important to consider that a modest elevation of tsRNA1 during early pregnancy might also represent a physiological adaptive response [76, 91, 92]. Therefore, future longitudinal studies are needed to establish baseline tsRNA1 levels and to clarify the thresholds that distinguish normal adaptive changes from those indicative of pathological stress.

Research implications

This study identifies tsRNA1 as a potential biomarker for early prediction of PTL, with its elevated levels in plasma extracellular vesicles linked to impaired EVT function. These findings underscore the potential of tsRNA1 analysis in improving prenatal risk assessment, enabling targeted monitoring for high-risk pregnancies. In contrast, while tsRNA3 is also upregulated in plasma extracellular vesicles, it does not appear to impact EVT function, and the reasons for its elevation remain unclear. And Further research is needed to understand the role of tsRNA3 and its potential implications in PTL mechanisms. Clinicians may need to consider the differential effects of these tsRNAs when assessing risk and developing management strategies for pregnant individuals.

Strengths and limitations

A key strength of this study is the systematic methodology encompassing the following critical steps:

1. Bioinformatics discovery phase: Re-analysis of public small RNA-seq datasets (PRJNA415953, PRJNA428989) to identify candidate tsRNAs.
2. Biomarker validation phase: Experimental validation of tsRNA1/tsRNA3 in a longitudinal cohort ($n = 83$) using qRT-PCR. And functional confirmation of tsRNA1's role in EVT dysfunction via HTR-8/SVneo cell-based assays.
3. Mechanistic exploration phase: Transcriptome profiling of tsRNA1-overexpressing EVT cells to identify dysregulated pathways. And validation of methylation-associated genes (e.g., DNMTs, TETs) at the mRNA level.

However, several limitations must be acknowledged. First, we exclusively used the HTR-8/SVneo cell line, which may not fully recapitulate primary EVT biology. Future studies with primary trophoblasts or in vivo models are needed to confirm translational relevance. Additionally, we humbly acknowledge that this study does not provide direct evidence of DNMT1-driven metabolic changes, such as SAM depletion or 5mC accumulation at specific genomic loci. Future work employing DNMT1 inhibitors (e.g., 5-azacytidine) and targeted metabolomics will be essential to validate this pathway. We are actively pursuing these experiments and welcome collaboration to address this gap. Furethermore, while we used TEM to visualize EVs and Western blotting for EV markers CD9 and CD63, we acknowledge that these methods alone cannot confirm the purity of the sample or rule out co-isolated contaminants. The absence of quantitative data, such as nanoparticle tracking analysis (NTA), limits our ability to rigorously assess the quality of the EV preparation. Therefore, caution is needed when assuming that all isolated particles are purely "extracellular vesicles" rather than exosomes.

Conclusion

In conclusion, our study highlights the critical role of plasma extracellular vesicle tsRNAs in the pathogenesis of PTL. The identification of tsRNA1 and tsRNA3 as predictive biomarkers significantly enhances the potential for early detection of PTL. While the mechanistic role of tsRNA1 in disease pathogenesis warrants further investigation, its association with dysregulated signaling pathways suggests possible therapeutic implications. Continued research is needed to determine whether tsRNA3, beyond its diagnostic value, contributes functionally to pregnancy-related complications. These findings underscore the importance of exploring tsRNAs as both diagnostic tools and potential mediators of pregnancy disorders.

Abbreviations

PTL	Preterm Labor
PTB	Preterm Birth
PPROM	Preterm Premature Rupture of Membranes
EVT	Extravillous Trophoblast
EV	Extracellular Vesicle
tsRNA	tRNA-derived Small RNA
PBMC	Peripheral Blood Mononuclear Cells
TEM	Transmission Electron Microscopy
AUC	Area Under the Curve
tRFs	tRNA-derived Fragments
TET1	Ten-eleven Translocation 1
DNMT1	DNA Methyltransferase 1
5mC	5-methylcytosine

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-025-07672-3.

Supplementary Material 1

Acknowledgements

We would like to express our sincere gratitude to the patients from West China Second University Hospital, Sichuan University, for their invaluable contributions in providing plasma samples for this research.

Author contributions

Xinrui Sun, Ying Feng, Liang Xie, and Yilun Wu these authors contributed equally as co-first authors. They were involved in the study design, data collection, and conducting experiments. JM, YZ, LZ, and XY contributed to the bioinformatics analysis, data interpretation, and manuscript drafting. JN provided technical assistance and critical revisions to the manuscript. XX and HL provided clinical expertise, coordinated sample collection, and reviewed the manuscript. LD and FM conceived and supervised the study, provided critical feedback, and finalized the manuscript. All authors read and approved the final version of the manuscript.

Funding

This study was funded by Sichuan Science and Technology Program (2024YFFK0325).

Data availability
The datasets analyzed during the current study are available from the corresponding author upon reasonable request. Publicly available GEO datasets used in this study include PRJNA415953 and PRJNA428989.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of West China Second Hospital (Approval No. k2019069 and k2022039). Written informed consent was obtained from all participants prior to sample collection and study participation, in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable, as no identifiable personal data of participants were included in the manuscript.

Competing interests
The authors declare no competing interests.

Author details
[1]Center for Translational Medicine, Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), West China Second University Hospital, Ministry of Education, Sichuan University, Chengdu, Sichuan 610041, P. R. China
[2]Department of Obstetrics and Gynecology, West China Second Hospital, Sichuan University, Chengdu, Sichuan 610041, China
[3]National Center for Birth Defects Monitoring, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, China
[4]National Health Commission Key Laboratory of Chronobiology (Sichuan University), Chengdu, Sichuan, China
[5]Key Laboratory of Birth Defects and Related Diseases of Women and Children, Sichuan University, Ministry of Education, Chengdu, China
[6]Department of Pediatric Pulmonology and Immunology, West China Second University Hospital, Sichuan University, Chengdu, China
[7]Department of Histology, Embryology and Neurobiology, West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu, Sichuan 610041, China
[8]The Joint Laboratory for Pulmonary Development and Related Diseases, West China Second University Hospital, West China Institute of Women and Children's Health, Sichuan University, Chengdu, Sichuan, China
[9]Key Laboratory of Bio-Resource and Eco-Environment of Ministry of Education, State Key Laboratory of Biotherapy and Cancer Center, College of Life Sciences, Sichuan University, Chengdu 610064, China
[10]College of Horticulture, Nanjing Agricultural University, Nanjing, Jiangsu, China

Received: 15 January 2025 / Accepted: 29 April 2025
Published online: 10 May 2025

References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371:75–84. https://doi.org/10.1016/s0140-6736(08)60074-4.
2. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345:760–5. https://doi.org/10.1126/science.1251816.
3. Cao G, Liu J, Liu M. Global, regional, and National incidence and mortality of neonatal preterm birth, 1990–2019. JAMA Pediatr. 2022;176:787–96. https://doi.org/10.1001/jamapediatrics.2022.1622.
4. The Lancet Respiratory, M. Improving lifelong respiratory health after preterm birth. Lancet Respir Med. 2022;10:121. https://doi.org/10.1016/s2213-2600(22)00018-2.
5. Ohuma EO, Moller AB, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, Okwaraji YB, Mahanani WR, Johansson EW, Lavin T, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. Lancet. 2023;402:1261–71. https://doi.org/10.1016/s0140-6736(23)00878-4.
6. Robbins CL, Hutchings Y, Dietz PM, Kuklina EV, Callaghan WM. History of preterm birth and subsequent cardiovascular disease: a systematic review. Am J Obstet Gynecol. 2014;210:285–97. https://doi.org/10.1016/j.ajog.2013.09.020.
7. de Eduardo P, de Rezende JAF, Menezes MG, P.R., and, Del-Ben CM. Preterm birth as a risk factor for postpartum depression: A systematic review and meta-analysis. J Affect Disord. 2019;259:392–403. https://doi.org/10.1016/j.jad.2019.08.069.
8. Markopoulou P, Papanikolaou E, Analytis A, Zoumakis E, Siahanidou T. Preterm birth as a risk factor for metabolic syndrome and cardiovascular disease in adult life: A systematic review and Meta-Analysis. J Pediatr. 2019;210:69–e8065. https://doi.org/10.1016/j.jpeds.2019.02.041.
9. Xu Y, Liu X, Zeng W, Zhu Y, Dong J, Wu F, Chen C, Sharma S, Lin Y. DOCK1 insufficiency disrupts trophoblast function and pregnancy outcomes via DUSP4-ERK pathway. Life Sci Alliance. 2024;7. https://doi.org/10.26508/lsa.202302247.
10. Turco MY, Moffett A. Development of the human placenta. Development. 2019;146. https://doi.org/10.1242/dev.163428.
11. Gao F, Zhou C, Qiu W, Wu H, Li J, Peng J, Qiu M, Liang C, Gao J, Luo S. Total flavonoids from semen cuscutae target MMP9 and promote invasion of EVT cells via Notch/AKT/MAPK signaling pathways. Sci Rep. 2018;8. https://doi.org/10.1038/s41598-018-35732-6.
12. Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. Placenta. 1983;4:397–413. https://doi.org/10.1016/s0143-4004(83)80043-5.
13. Varberg KM, Iqbal K, Muto M, Simon ME, Scott RL, Kozai K, Choudhury RH, Aplin JD, Biswell R, Gibson M, et al. ASCL2 reciprocally controls key trophoblast lineage decisions during hemochorial placenta development. Proc Natl Acad Sci U S A. 2021;118. https://doi.org/10.1073/pnas.2016517118.
14. Sarker S, Scholz-Romero K, Perez A, Illanes SE, Mitchell MD, Rice GE, Salomon C. Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J Transl Med. 2014;12:204. https://doi.org/10.1186/1479-5876-12-204.
15. Weingrill RB, Paladino SL, Souza MLR, Pereira EM, Marques ALX, Silva ECO, da Silva Fonseca EJ, Ursulino JS, Aquino TM, Bevilacqua E, et al. Exosome-Enriched plasma analysis as a tool for the early detection of hypertensive gestations. Front Physiol. 2021;12:767112. https://doi.org/10.3389/fphys.2021.767112.
16. Mitchell MD, Peiris HN, Kobayashi M, Koh YQ, Duncombe G, Illanes SE, Rice GE, Salomon C. Placental exosomes in normal and complicated pregnancy. Am J Obstet Gynecol. 2015;213:S173–181. https://doi.org/10.1016/j.ajog.2015.07.001.
17. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, Cai H, Di Vizio D, Driedonks TAP, Erdbrügger U, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J Extracell Vesicles. 2024;13:e12404. https://doi.org/10.1002/jev2.12404.
18. Chen S, Zhang X, Meng K, Sun Y, Shu R, Han Y, Feng Q, Li Z, Yang P, Liang J. Urinary exosome TsRNAs as novel markers for diagnosis and prediction of lupus nephritis. Front Immunol. 2023;14:1077645. https://doi.org/10.3389/fimmu.2023.1077645.
19. Xu W, Yu M, Wu Y, Jie Y, Li X, Zeng X, Yang F, Chong Y. Plasma-Derived Exosomal SncRNA as a promising diagnostic biomarker for early detection of HBV-Related Acute-on-Chronic liver failure. Front Cell Infect Microbiol. 2022;12:923300. https://doi.org/10.3389/fcimb.2022.923300.
20. Gu X, Zhang Y, Qin X, Ma S, Huang Y, Ju S. Transfer RNA-derived small RNA: an emerging small non-coding RNA with key roles in cancer. Exp Hematol Oncol. 2022;11:35. https://doi.org/10.1186/s40164-022-00290-1.
21. Li X, Liu X, Zhao D, Cui W, Wu Y, Zhang C, Duan C. tRNA-derived small RNAs: novel regulators of cancer hallmarks and targets of clinical application. Cell Death Discov. 2021;7:249. https://doi.org/10.1038/s41420-021-00647-1.
22. Panoutsopoulou K, Magkou P, Dreyer T, Dorn J, Obermayr E, Mahner S, van Gorp T, Braicu I, Magdolen V, Zeillinger R, et al. tRNA-derived small RNA 3'U-tRF(ValCAC) promotes tumour migration and early progression in ovarian cancer. Eur J Cancer. 2023;180:134–45. https://doi.org/10.1016/j.ejca.2022.11.033.
23. Hu F, Niu Y, Mao X, Cui J, Wu X, Simone CB 2nd, Kang HS, Qin W, Jiang L. tsRNA-5001a promotes proliferation of lung adenocarcinoma cells and is associated with postoperative recurrence in lung adenocarcinoma patients. Transl Lung Cancer Res. 2021;10:3957–72. https://doi.org/10.21037/tlcr-21-829.
24. Xiong Q, Zhang Y, Xu Y, Yang Y, Zhang Z, Zhou Y, Zhang S, Zhou L, Wan X, Yang X, et al. tiRNA-Val-CAC-2 interacts with FUBP1 to promote

pancreatic cancer metastasis by activating c–MYC transcription. Oncogene. 2024;43:1274–87. https://doi.org/10.1038/s41388-024-02991-9.
25. Camunas-Soler J, Gee EPS, Reddy M, Mi JD, Thao M, Brundage T, Siddiqui F, Hezelgrave NL, Shennan AH, Namsaraev E, et al. Predictive RNA profiles for early and very early spontaneous preterm birth. Am J Obstet Gynecol. 2022;227. https://doi.org/10.1016/j.ajog.2022.04.002.
26. Wallenstein MB, Jelliffe-Pawlowski LL, Yang W, Carmichael SL, Stevenson DK, Ryckman KK, Shaw GM. Inflammatory biomarkers and spontaneous preterm birth among obese women. J Matern Fetal Neonatal Med. 2016;29:3317–22. https://doi.org/10.3109/14767058.2015.1124083.
27. Ward VC, Hawken S, Chakraborty P, Darmstadt GL, Wilson K. Estimating gestational age and prediction of preterm birth using metabolomics biomarkers. Clin Perinatol. 2024;51:411–24. https://doi.org/10.1016/j.clp.2024.02.012.
28. Bastek JA, Elovitz MA. The role and challenges of biomarkers in spontaneous preterm birth and preeclampsia. Fertil Steril. 2013;99:1117–23. https://doi.org/10.1016/j.fertnstert.2013.01.104.
29. Beernink RHJ, Schuitemaker JHN, Zwertbroek EF, Scherjon SA, Cremers T. Early pregnancy biomarker discovery study for spontaneous preterm birth. Placenta. 2023;139:112–9. https://doi.org/10.1016/j.placenta.2023.06.011.
30. Illarionov RA, Pachuliia OV, Vashukova ES, Tkachenko AA, Maltseva AR, Postnikova TB, Nasykhova YA, Bespalova ON, Glotov AS. Plasma MiRNA profile in high risk of preterm birth during early and Mid-Pregnancy. Genes (Basel). 2022;13. https://doi.org/10.3390/genes13112018.
31. Weiner CP, Dong Y, Zhou H, Cuckle H, Ramsey R, Egerman R, Buhimschi I, Buhimschi C. Early pregnancy prediction of spontaneous preterm birth before 32 completed weeks of pregnancy using plasma RNA: transcriptome discovery and initial validation of an RNA panel of markers. BJOG. 2021;128:1870–80. https://doi.org/10.1111/1471-0528.16736.
32. Pan Q, Han T, Li G. Novel insights into the roles of tRNA-derived small RNAs. RNA Biol. 2021;18:2157–67. https://doi.org/10.1080/15476286.2021.1922009.
33. Abbas Y, Turco MY, Burton GJ, Moffett A. Investigation of human trophoblast invasion in vitro. Hum Reprod Update. 2020;26:501–13. https://doi.org/10.1093/humupd/dmaa017.
34. Dunk CE, van Dijk M, Choudhury R, Wright TJ, Cox B, Leavey K, Harris LK, Jones RL, Lye SJ. Functional evaluation of STOX1 (STORKHEAD-BOX PROTEIN 1) in placentation, preeclampsia, and preterm birth. Hypertension. 2021;77:475–90. https://doi.org/10.1161/hypertensionaha.120.15619.
35. Tiensuu H, Haapalainen AM, Tissarinen P, Pasanen A, Määttä TA, Huusko JM, Ohlmeier S, Bergmann U, Ojaniemi M, Muglia LJ, et al. Human placental proteomics and exon variant studies link AAT/SERPINA1 with spontaneous preterm birth. BMC Med. 2022;20. https://doi.org/10.1186/s12916-022-02339-8.
36. Graham CH, Hawley TS, Hawley RG, MacDougall JR, Kerbel RS, Khoo N, Lala PK. Establishment and characterization of first trimester human trophoblast cells with extended lifespan. Exp Cell Res. 1993;206:204–11. https://doi.org/10.1006/excr.1993.1139.
37. Hu H, Ma J, Peng Y, Feng R, Luo C, Zhang M, Tao Z, Chen L, Zhang T, Chen W, et al. Thrombospondin-1 regulates trophoblast necroptosis via NEDD4-Mediated ubiquitination of TAK1 in preeclampsia. Adv Sci (Weinh). 2024;11:e2309002. https://doi.org/10.1002/advs.202309002.
38. Jeyarajah MJ, Jaju Bhattad G, Kelly RD, Baines KJ, Jaremek A, Yang FP, Okae H, Arima T, Dumeaux V, Renaud SJ. The multifaceted role of GCM1 during trophoblast differentiation in the human placenta. Proc Natl Acad Sci U S A. 2022;119:e2203071119. https://doi.org/10.1073/pnas.2203071119.
39. Shao X, Cao G, Chen D, Liu J, Yu B, Liu M, Li YX, Cao B, Sadovsky Y, Wang YL. Placental trophoblast syncytialization potentiates macropinocytosis via mTOR signaling to adapt to reduced amino acid supply. Proc Natl Acad Sci U S A. 2021;118. https://doi.org/10.1073/pnas.2017092118.
40. Pique-Regi R, Romero R, Tarca AL, Sendler ED, Xu Y, Garcia-Flores V, Leng Y, Luca F, Hassan SS, Gomez-Lopez N. Single cell transcriptional signatures of the human placenta in term and preterm parturition. Elife. 2019;8. https://doi.org/10.7554/eLife.52004
41. Straszewski-Chavez SL, Abrahams VM, Mor G. The role of apoptosis in the regulation of trophoblast survival and differentiation during pregnancy. Endocr Rev. 2005;26:877–97. https://doi.org/10.1210/er.2005-0003.
42. Takeshima H, Niwa T, Yamashita S, Takamura-Enya T, Iida N, Wakabayashi M, Nanjo S, Abe M, Sugiyama T, Kim YJ, Ushijima T. TET repression and increased DNMT activity synergistically induce aberrant DNA methylation. J Clin Invest. 2020;130:5370–9. https://doi.org/10.1172/jci124070.
43. Tsiouplis NJ, Bailey DW, Chiou LF, Wissink FJ, Tsagaratou A. TET-Mediated epigenetic regulation in immune cell development and disease. Front Cell Dev Biol. 2020;8:623948. https://doi.org/10.3389/fcell.2020.623948.
44. Andrews S, Krueger C, Mellado-Lopez M, Hemberger M, Dean W, Perez-Garcia V, Hanna CW. Mechanisms and function of de Novo DNA methylation in placental development reveals an essential role for DNMT3B. Nat Commun. 2023;14. https://doi.org/10.1038/s41467-023-36019-9.
45. Huang W, Chen ACH, Wei X, Fong SW, Yeung WSB, Lee YL. Uncovering the role of TET2-mediated ENPEP activation in trophoblast cell fate determination. Cell Mol Life Sci. 2024;81:270. https://doi.org/10.1007/s00018-024-05306-z.
46. Kusuyama J, Alves-Wagner AB, Conlin RH, Makarewicz NS, Albertson BG, Prince NB, Kobayashi S, Kozuka C, Møller M, Bjerre M, et al. Placental superoxide dismutase 3 mediates benefits of maternal exercise on offspring health. Cell Metab. 2021;33:939–e956938. https://doi.org/10.1016/j.cmet.2021.03.004.
47. Ville Y, Rozenberg P. Predictors of preterm birth. Best Pract Res Clin Obstet Gynaecol. 2018;52:23–32. https://doi.org/10.1016/j.bpobgyn.2018.05.002.
48. Kindschuh WF, Baldini F, Liu MC, Liao J, Meydan Y, Lee HH, Heinken A, Thiele I, Thaiss CA, Levy M, Korem T. Preterm birth is associated with xenobiotics and predicted by the vaginal metabolome. Nat Microbiol. 2023;8:246–59. https://doi.org/10.1038/s41564-022-01293-8.
49. Gillespie SL, Christian LM, Mackos AR, Nolan TS, Gondwe KW, Anderson CM, Hall MW, Williams KP, Slavich GM. Lifetime stressor exposure, systemic inflammation during pregnancy, and preterm birth among black American women. Brain Behav Immun. 2022;101:266–74. https://doi.org/10.1016/j.bbi.2022.01.008.
50. Chen Z, Li J, Xu W, Wu X, Xiang F, Li X, Zhang M, Zheng J, Kang X, Wu R. Elevated expression of Toll-like receptor 4 and cytokines in both serum and myometrium at term May serve as promising biomarkers for uterine activation preceding labor. Front Endocrinol (Lausanne). 2023;14:1255925. https://doi.org/10.3389/fendo.2023.1255925.
51. Vora N, Kalagiri R, Mallett LH, Oh JH, Wajid U, Munir S, Colon N, Raju VN, Beeram MR, Uddin MN. Proteomics and metabolomics in Pregnancy-An overview. Obstet Gynecol Surv. 2019;74:111–25. https://doi.org/10.1097/ogx.0000000000000646.
52. Siristatidis CS, Sertedaki E, Vaidakis D. Metabolomics for improving pregnancy outcomes in women undergoing assisted reproductive technologies. Cochrane Database Syst Rev. 2017;5. Cd011872.
53. McCabe CF, Perng W. Metabolomics of diabetes in pregnancy. Curr Diab Rep. 2017;17. https://doi.org/10.1007/s11892-017-0890-3.
54. Wei SQ, Fraser W, Luo ZC. Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review. Obstet Gynecol. 2010;116:393–401. https://doi.org/10.1097/AOG.0b013e3181e6dbc0.
55. Wang J, Ma G, Li M, Han X, Xu J, Liang M, Mao X, Chen X, Xia T, Liu X, Wang S. Plasma tRNA fragments derived from 5' ends as novel diagnostic biomarkers for Early-Stage breast Cancer. Mol Ther Nucleic Acids. 2020;21:954–64. https://doi.org/10.1016/j.omtn.2020.07.026.
56. Zhu L, Li J, Gong Y, Wu Q, Tan S, Sun D, Xu X, Zuo Y, Zhao Y, Wei YQ, et al. Exosomal tRNA-derived small RNA as a promising biomarker for cancer diagnosis. Mol Cancer. 2019;18. https://doi.org/10.1186/s12943-019-1000-8.
57. Giannubilo SR, Cecati M, Marzioni D, Ciavattini A. Circulating MiRNAs and preeclampsia: from implantation to epigenetics. Int J Mol Sci. 2024;25. https://doi.org/10.3390/ijms25031418.
58. Jin M, Ji J, Chen X, Zhou Y, Wang D, Liu A. The emerging role of TET enzymes in the immune microenvironment at the maternal-fetal interface during decidualization and early pregnancy. Front Immunol. 2022;13:1066599. https://doi.org/10.3389/fimmu.2022.1066599.
59. Meng Y, Meng Y, Li L, Li Y, He J, Shan Y. The role of DNA methylation in placental development and its implications for preeclampsia. Front Cell Dev Biol. 2024;12:1494072. https://doi.org/10.3389/fcell.2024.1494072.
60. Jiang X, Li L. Decidual macrophage: a reversible role in immunotolerance between mother and fetus during pregnancy. Arch Gynecol Obstet. 2024;309:1735–44. https://doi.org/10.1007/s00404-023-07364-3.
61. Timofeeva AV, Fedorov IS, Tarasova AM, Sukhova YV, Kolod'ko VG, Ivanets TY, Sukhikh GT. Universal First-Trimester screening biomarkers for diagnosis of preeclampsia and placenta accreta spectrum. Biomolecules. 2025;15. https://doi.org/10.3390/biom15020228.
62. Gao Y, Huang Y, Guo K, Cheng J, Luo Y, Deng Y, Lei M. Advances in research on the mechanism of TsRNA action in tumours. J Med Genet. 2025;62:152–9. https://doi.org/10.1136/jmg-2024-110437.
63. Zang X, Zhang D, Wang W, Ding Y, Wang Y, Gu S, Shang Y, Gan J, Jiang L, Meng F, et al. Cross-Species insights into trophoblast invasion during placentation governed by Immune-Featured trophoblast cells. Adv Sci (Weinh). 2024;11:e2407221. https://doi.org/10.1002/advs.202407221.

64. Mora-Palazuelos C, Villegas-Mercado CE, Avendaño-Félix M, Lizárraga-Verdugo E, Romero-Quintana JG, López-Gutiérrez J, Beltrán-Ontiveros S, Bermúdez M. The role of NcRNAs in the immune dysregulation of preeclampsia. Int J Mol Sci. 2023;24. https://doi.org/10.3390/ijms242015215.
65. Jianfeng M, Mailin G, Yiting Y, Lei C, Ye Z, Lili N, Yan W, Shunhua Z, Jingyong W, Li Z, Linyuan S. tRNA-derived small RNA dataset in multiple organs of intrauterine growth-restricted pig. Sci Data. 2023;10. https://doi.org/10.1038/s41597-023-02715-w.
66. Xing C, Yang F, Lin Y, Shan J, Yi X, Ali F, Zhu Y, Wang C, Zhang C, Zhuang Y, et al. Hexavalent chromium exposure induces intestinal barrier damage via activation of the NF-κB signaling pathway and NLRP3 inflammasome in ducks. Front Immunol. 2022;13:952639. https://doi.org/10.3389/fimmu.2022.952639.
67. Ying S, Li P, Wang J, Chen K, Zou Y, Dai M, Xu K, Feng G, Zhang C, Jiang H, et al. tRF-Gln-CTG-026 ameliorates liver injury by alleviating global protein synthesis. Signal Transduct Target Ther. 2023;8. https://doi.org/10.1038/s41392-023-01351-5.
68. He Y, Wang W, Jiang J, Shen Y, Wang B, Chen J, Li M, Xu H. Infliximab inhibits TNF-α-dependent activation of the NLRP3/IL-1β pathway in acne inversa. Heliyon. 2024;10:e33146. https://doi.org/10.1016/j.heliyon.2024.e33146.
69. Han XY, Kong LJ, Li D, Tong M, Li XM, Zhao C, Jiang Q, Yan B. Targeting endothelial glycolytic reprogramming by tsRNA-1599 for ocular anti-angiogenesis therapy. Theranostics. 2024;14:3509–25. https://doi.org/10.7150/thno.96946.
70. Zuo Y, Zhu L, Guo Z, Liu W, Zhang J, Zeng Z, Wu Q, Cheng J, Fu X, Jin Y, et al. TsRBase: a comprehensive database for expression and function of TsRNAs in multiple species. Nucleic Acids Res. 2021;49:D1038–45. https://doi.org/10.1093/nar/gkaa888.
71. Shi J, Zhang Y, Zhou T, Chen Q. TsRNAs: the Swiss army knife for translational regulation. Trends Biochem Sci. 2019;44:185–9. https://doi.org/10.1016/j.tibs.2018.09.007.
72. Wu Y, Yang X, Jiang G, Zhang H, Ge L, Chen F, Li J, Liu H, Wang H. 5′-tRF-GlyGCC: a tRNA-derived small RNA as a novel biomarker for colorectal cancer diagnosis. Genome Med. 2021;13:20. https://doi.org/10.1186/s13073-021-00833-x.
73. Wen JT, Huang ZH, Li QH, Chen X, Qin HL, Zhao Y. Research progress on the TsRNA classification, function, and application in gynecological malignant tumors. Cell Death Discov. 2021;7:388. https://doi.org/10.1038/s41420-021-00789-2.
74. Ray S, Saha A, Ghosh A, Roy N, Kumar RP, Meinhardt G, Mukerjee A, Gunewardena S, Kumar R, Knöfler M, Paul S. Hippo signaling cofactor, WWTR1, at the crossroads of human trophoblast progenitor self-renewal and differentiation. Proc Natl Acad Sci U S A. 2022;119:e2204069119. https://doi.org/10.1073/pnas.2204069119.
75. Schatz F, Guzeloglu-Kayisli O, Arlier S, Kayisli UA, Lockwood CJ. The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. Hum Reprod Update. 2016;22:497–515. https://doi.org/10.1093/humupd/dmw004.
76. Fu X, Li Y, Zhang Z, Wang B, Wei R, Chu C, Xu K, Li L, Liu Y, Li X. Emerging role of MiRNAs, LncRNAs, and circrnas in pregnancy-associated diseases. Chin Med J (Engl). 2023;136:1300–10. https://doi.org/10.1097/cm9.0000000000002595.
77. Liu B, Liu J, Qiu Y, Chen J, Yang J. MITA promotes macrophage Proinflammatory polarization and its circRNA-Related regulatory mechanism in recurrent miscarriage. Int J Mol Sci. 2023;24. https://doi.org/10.3390/ijms24119545.
78. Wang L, Yin Z, Shen Y, Feng G, Dai F, Yang D, Deng Z, Yang J, Chen R, Yang L, et al. Targeting decidual CD16(+) immune cells with Exosome-Based glucocorticoid nanoparticles for miscarriage. Adv Sci (Weinh). 2024;e2406370. https://doi.org/10.1002/advs.202406370.
79. Tian H, Hu Z, Wang C. The therapeutic potential of tRNA-derived small RNAs in neurodegenerative disorders. Aging Dis. 2022;13:389–401. https://doi.org/10.14336/ad.2021.0903.
80. Li K, Lin Y, Luo Y, Xiong X, Wang L, Durante K, Li J, Zhou F, Guo Y, Chen S, et al. A signature of saliva-derived Exosomal small RNAs as predicting biomarker for esophageal carcinoma: a multicenter prospective study. Mol Cancer. 2022;21. https://doi.org/10.1186/s12943-022-01499-8.
81. Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, Feng GH, Peng H, Zhang X, Zhang Y, et al. Sperm TsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science. 2016;351:397–400. https://doi.org/10.1126/science.aad7977.
82. Wu Z, Zhou R, Li B, Cao M, Wang W, Li X. Methylation modifications in tRNA and associated disorders: current research and potential therapeutic targets. Cell Prolif. 2024;e13692. https://doi.org/10.1111/cpr.13692.
83. Xiong Q, Zhang Y. Small RNA modifications: regulatory molecules and potential applications. J Hematol Oncol. 2023;16:64. https://doi.org/10.1186/s13045-023-01466-w.
84. Sobala A, Hutvagner G. Small RNAs derived from the 5′ end of tRNA can inhibit protein translation in human cells. RNA Biol. 2013;10:553–63. https://doi.org/10.4161/rna.24285.
85. Branco MR, King M, Perez-Garcia V, Bogutz AB, Caley M, Fineberg E, Lefebvre L, Cook SJ, Dean W, Hemberger M, Reik W. Maternal DNA methylation regulates early trophoblast development. Dev Cell. 2016;36:152–63. https://doi.org/10.1016/j.devcel.2015.12.027.
86. Yang YF, Li ZH, Liu TH, Li C, Xie YL, Ge LX, Tang J, Li FF, Luo X, Fu LJ, Ding YB. Evaluating the role of DNA methyltransferases in trophoblast fusion and preeclampsia development: insights from methylation-regulated genes. Faseb J. 2024;38(e23706). https://doi.org/10.1096/fj.202302705R.
87. Chrysanthou S, Senner CE, Woods L, Fineberg E, Okkenhaug H, Burge S, Perez-Garcia V, Hemberger M. A critical role of TET1/2 proteins in Cell-Cycle progression of trophoblast stem cells. Stem Cell Rep. 2018;10:1355–68. https://doi.org/10.1016/j.stemcr.2018.02.014.
88. Brombach C, Tong W, Giussani DA. Maternal obesity: new placental paradigms unfolded. Trends Mol Med. 2022;28:823–35. https://doi.org/10.1016/j.molmed.2022.05.013.
89. Xu Y, Qin X, Zeng W, Wu F, Wei X, Li Q, Lin Y. DOCK1 deficiency drives placental trophoblast cell dysfunction by influencing inflammation and oxidative stress, hallmarks of preeclampsia. Hypertens Res. 2024;47:3434–46. https://doi.org/10.1038/s41440-024-01920-3.
90. Huo W, Wang Y, Chen T, Cao T, Zhang Y, Shi Z, Hou S. Triclosan activates c-Jun/miR-218-1-3p/SLC35C1 signaling to regulate cell viability, migration, invasion and inflammatory response of trophoblast cells in vitro. BMC Pregnancy Childbirth. 2022;22. https://doi.org/10.1186/s12884-022-04791-z.
91. Mercer GV, Stapleton D, Barrett C, Ringer LCM, Lambe S, Critch A, Newman G, Pelley A, Biswas RG, Wolff W, et al. Identifying placental metabolic biomarkers of preterm birth using nuclear magnetic resonance of intact tissue samples. Placenta. 2023;143:80–6. https://doi.org/10.1016/j.placenta.2023.10.006.
92. Qiao L, Lu C, Zang T, Dzyuba B, Shao J. Maternal GLP-1 receptor activation inhibits fetal growth. Am J Physiol Endocrinol Metab. 2024;326:E268–76. https://doi.org/10.1152/ajpendo.00361.2023.

Publisher's note