Effects of histone H4 hyperacetylation on inhibiting MMP2 and MMP9 in human amniotic epithelial cells and in premature rupture of fetal membranes

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Abstract. Histone modification is closely associated with several diseases. The aim of the current study was to investigate the associations among histone acetylation, matrix metalloproteinases (MMPs) and premature rupture of membranes (PROM) during pregnancy. A total of 180 puerperants were divided into three groups: i) Preterm-PROM (PPROM), ii) term-PROM (TPROM) and iii) full-term labor (FTL). Enzyme-linked immunosorbent assay (ELISA) kits and western blotting were used to determine the protein concentrations of MMP2, MMP9, histone deacetylase (HDAC)1, HDAC2 and HDAC6, and the protein levels of histone H4 lysine (H4K)5 and H4K8 acetylation, respectively, in three types of fetal membranes. Additionally, human amniotic epithelial cells were used to determine the effects of the HDAC inhibitors droxinostat and chidamide on cell viability, histone acetylation and the levels of MMP2, MMP9, HDAC1, HDAC2 and HDAC6 *in vitro*, using the Cell Counting Kit-8 assay, western blotting and ELISA, respectively. Furthermore, the effects of droxinostat and chidamide on the invasion and migration abilities of human amniotic epithelial cells were investigated using transwell assays. In fetal membranes, the activities of MMP2 and MMP9 increased in PPROM, but decreased in TPROM. Further, the expression of HDAC1 was decreased and histone hyperacetylation was increased in both PPROM and TRPOM. *In vitro* experiments revealed that 5 μM droxinostat and 0.5 μM chidamide selectively decreased the level of HDAC and induced acetylation of H4K5 and H4K8. Additionally, the aforementioned HDAC inhibitors reduced human amniotic epithelial cell viability, invasion and migration, and decreased the expression levels of MMP2 and MMP9. The current study revealed a high expression level of MMP2 and MMP9 in PPROM compared with TPROM and FL tissue, which was in accordance with previously published studies. Furthermore, the *in vitro* tests performed in the current study revealed the effect of histone H4 hyperacetylation on inhibiting MMP2 and MMP9 levels *in vitro* was similar to that observed in TPROM. The results obtained in the current study may be used as a theoretical guide for clinical treatment of premature rupture of membranes.

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Introduction

Premature rupture of membranes (PROM) refers to a spontaneous rupture of fetal membranes that occurs at least 2 h prior to the onset of labor. PROM that occurs in patients with >37 weeks gestation is referred to as term premature rupture of membranes (TPROM). Preterm premature rupture of membranes (PPROM) occurs at <37 weeks gestation and has an incidence of ~3%, accounting for approximately one third of preterm births in Nigeria during 1993-2003 (1). PROM is often associated with puerperal and neonatal infection, fetal malformation, premature infants and maternal complications (2-6). Obstetric disease often occurs due to multiple risk factors, including amnionitis, multifetal pregnancy, polyhydramnios, cervical relaxation or surgery, trauma, maternal smoking status, poor maternal nutrition, abnormal pH values, congenital amniotic dysplasia, hereditary amniotic membrane thinning, and vitamin C, trace element zinc and copper deficiency (7-10). Fetal membranes consist of chorion, amniotic membrane, basement membrane and extracellular matrix (ECM) (11,12). The nature of the membrane structure allows PROM to cause the membranes to become thin and brittle, and eventually rupture (13).

Matrix metalloproteinases (MMPs) are zinc and calcium-dependent proteolytic enzymes that participate in the degradation of ECM. MMPs are divided into six categories as follows: Collagenase, stromal lysin, gelatinase, membrane metalloproteinase, matrix enzyme and secreted MMP (14). The MMP family is involved in physiological and pathological

processes, and has attracted attention in the field of gynecological and obstetric diseases (15,16). MMPs are located between the chorionic and amniotic membranes, and the enzymes MMP9, MMP2, MMP13 and MMP8 may serve a key role in the rupture of the fetal membranes (17-20).

The histone octamer consists of two copies of each histone, including H2A, H2B, H3 and H4, which are involved in DNA organization and folding in the nucleus (21). Acetylation modification is an important mechanism by which DNA may be unfolded and it is a reversible dynamic process (22). Histone acetylation is largely catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which activate and inhibit gene transcription, respectively (22). HDAC inhibitors are a class of agents designed to block HDACs, therefore allowing HATs to induce histone acetylation and to promote gene transcription (23). The structure of four core histones (H2A, H2B, H3 and H4) and their associated modifications are associated with gynecologic and obstetric diseases (24).

MMPs serve an important role in mediating degradation of the ECM in pathologic conditions such as PROM (25,26). In addition, Poljak *et al* (27) suggested that HDACs regulate MMP-9 expression in primary amnion cells. The current study employed the HDAC inhibitors droxinostat and chidamide to block HDACs and to investigate whether the levels of histone acetylation are associated with MMPs in human amniotic epithelial cells.

Materials and methods

Patients and tissue samples. Patients with PROM were diagnosed according to the American College of Obstetricians and Gynecologists (ACOG) guidelines on the diagnosis and treatment of PROM (version 2013) (28). The diagnostic criteria were as follows: i) Leaking of amniotic fluid prior to labor, ii) an alkaline pH test and iii) a positive amniotic fluid crystallization test. A total of 7,630 puerperant, among them 4,230 who were spontaneously delivered, were identified between May 2016 and March 2017 at the Maternal and Child Health Hospital of Tangshan (Tangshan, China). A total of 180 puerperants were selected using the following inclusion criteria: i) Patients agreed to give birth naturally, ii) single fetus with head-first presentation, iii) patients conformed with the aforementioned ACOG diagnostic criteria, iv) patients delivered within 48 h following admission, v) patients with normal body temperature on admission, vi) no mechanical interventions used during pregnancy and vii) patients without complications such as hypertension, diabetes, heart disease and asthma. Patients were divided into three groups, PPROM, TPROM and full-term labor (FTL), depending on whether the duration of gestation exceeded 37 weeks prior to membrane rupture. The basic information of the patients is presented in Table I. The current study was approved by the Ethics Committee of the Maternal and Child Health Hospital of Tangshan (Tangshan, China) and written informed consent was obtained from each patient.

Approximately 10 g of fetal membrane tissue was cut close to the area of rupture from which amniotic fluid was released within 15 min of natural delivery. The tissues collected were rinsed three to four times in physiological saline solution containing 0.9% sodium chloride and stored at -40˚C.

Cell culture. Human amniotic epithelial (HAEpi) cells were obtained from Sciencell Research Laboratories, Inc. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin/streptomycin (all Thermo Fisher Scientific, Inc.) and 2 mM glutamine at 37˚C and 5% CO_2. The cells were grown to 80% confluence and trypsinized using phosphate-buffered saline (PBS) containing 0.25% trypsin (29).

Immunofluorescence staining. Immunofluorescence staining was used to verify the presence of HAEpi cells, which were seeded in six-well plates ($2x10^5$ cells/well) and fixed with 4% paraformaldehyde at room temperature for 20 min. Cell were subsequently incubated with PBS containing 0.3% Triton X-100 at 4˚C for 15 min. The cells were blocked with 10% normal goat serum (cat. no. ab7481; 1:50; Abcam) at 37˚C for 20 min and then incubated with an anti-cytokeratin (CK)-19 antibody (cat. no. ab15463; 1:100; Abcam) at 4˚C overnight (30). Cells were washed using PBS and incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (cat. no. 10285-1-AP; 1:1,000; ProteinTech Group, Inc.) at room temperature for 1 h. Nuclei were counterstained using 4',6-diamidino-2-phenylindole (DAPI) at room temperature for 10 min. An anti-fluorescence quenching agent (ProLong® Antifade kit; cat. no. P7481; Thermo Fisher Scientific, Inc.) was then added to cells in the dark at room temperature for 30 min. Images were subsequently taken using a fluorescence microscope (magnification, x400) with NIS-Elements BR software version 4.60.

Treating cells with HDAC inhibitors. The HDAC inhibitors droxinostat (cat. no. S1422) and chidamide (cat. no. S8567) were purchased from Selleck Chemicals. In order to determine the effects of the HDAC inhibitors on cell viability, HAEpi cells were exposed to droxinostat (0, 1, 5, 10 and 20 μM) and chidamide (0, 0.1, 0.2, 0.5 and 1 μM) and dissolved in DMSO at room temperature for 24, 48 and 72 h. For other *in vitro* experiments (detection of H4K5ac and H4K8ac protein expression and the detection of MMP-2, MMP-9 and HDAC-1, 2 and 6), HAEpi cells were treated with the DMSO (control) or with HDAC inhibitors, droxinostat (5 μM) and chidamide (0.5 μM), for 48 h to assess other *in vitro* experiments.

Cell Counting Kit-8 (CCK-8) assay. HAEpi cells were plated into 96-well plates at a seeding density of $1x10^4$ cells per well and incubated at 37˚C for 24 h. The cells were exposed to the HDAC inhibitors at the aforementioned concentrations for 24, 48 and 72 h. A total of 10 μl CCK-8 solution was subsequently added to each well and incubated for an additional 3 h at 37˚C. Cell viability was determined by measuring the optical density at a wavelength of 450 nm using a microplate reader.

Enzyme-linked immunosorbent assay (ELISA). The concentrations of MMP2, MMP9, HDAC1, HDAC2 and HDAC6 in fetal membrane tissues and HAEpi cells were measured using ELISA kits. A total of 100 mg of fetal membrane tissues were homogenized in 1 ml protein lysate buffer (comprising 2.5 ml Triton X and one tablet of Roche Complete EDTA-free Protease Inhibitor (Sigma-Aldrich; Merck KGaA) to a volume

Table I. Basic clinical information of the patients enrolled in the current study.

Variable	PPROM (n=60)	TPROM (n=60)	FTL (n=60)	P-value
Age	28.26±5.13	27.79±3.44	27.5±3.67	0.600
BMI	28.95±5.24[a,b]	26.98±3.71	27.28±4.26	0.035
Gestational age (week)	33.87±1.83	39.06±1.90	39.65±0.93	<0.001
TMRD (h)	38.6±59.05	21.93±22.27	0.00	<0.001
One-child ratio	71.67%	76.67%	75.00%	0.815
IAH	0.00%	8.33%	80.00%	<0.001
Eosinophils (%)	0.92±0.28	1.03±1.74	1.36±0.38	0.057
Neutrophils (%)	75.65±11.56[a]	74.11±10.51[a]	71.34±13.85	0.143
Glucose (mmol/l)	6.06±1.72	5.84±1.73	6.13±1.87	0.647
Albumin (g/l)	31.84±5.56[a]	33.22±5.31[a]	35.52±6.65	0.003

The Student-Newman-Keuls test was used to compare between each of the three sets of data. [a]P<0.05 vs. FTL group and [b]P<0.05 vs. TPROM group. PROM, premature rupture of membranes; PPROM, pre-term PROM; TPROM, term PROM; FTL, full-term labor; BMI, body mass index; TMRD, time of membrane rupture to delivery; IAH, incidence of antepartum hemorrhage. The normal ranges for eosinophil is <10.0%; The normal ranges for neutrophils is 50-70%; Glucose normal range, 3.9-6.1 mmol/l; Albumin normal range, 35.0-52.0 g/l; One-child ratio, number of babies born in the current study as the first baby of the mother/the number of all parturients included in the present study x100%.

of 50 ml), centrifuged at 4,042 x g at room temperature for 10 min and supernatants were collected. HAEpi cells ($1x10^6$ cells/well) were seeded on a 24-well plate at 37˚C. Cell-free culture media harvested after 3 h. ELISA kits were used to detect the concentrations of MMP2 (cat. no. MMP-200), MMP9 (cat. no. DMP-900; both R&D Systems, Inc.), HDAC1 (cat. no. ml037173; Shanghai Enzyme-linked Biotechnology Co., Ltd), HDAC2 (cat. no. IT4052; G-Biosciences), HDAC6 (cat. no. IT4738; G-Biosciences) in the culture media according to the manufacturer's instructions and as described previously (31,32).

Transwell assay. Cell migration and invasion were performed using uncoated and Matrigel-coated transwell respectively. Following exposure to droxinostat (5 μM), chidamide (0.5 μM) or DMSO (control group; 5 μM or 0.5 μM) for 48 h, HAEpi cells were resuspended in serum-free DMEM, and $1x10^4$ cells were added into the upper chamber of the transwell insert (Corning, Inc.). DMEM supplemented with 10% FBS was added to the lower chamber and the cells were incubated at 37˚C for 24 h. The cells were subsequently fixed with 1% formaldehyde for 10 min at room temperature and stained with 0.5% crystal violet at room temperature for 5 min after the Matrigel coated filter membrane was removed. The number of migratory cells in five randomly selected fields were imaged using a light microscope (magnification, x200) and counted using ImageJ software (Version 1.49; National Institutes of Health).

Western blot analysis. Proteins were extracted from fetal membrane tissues or HAEpi cells using radioimmunoprecipitation assay buffer (Thermo Fisher Scientific, Inc.). Total protein was quantified using a bicinchoninic acid kit (Beyotime Institute of Biotechnology). Aliquots of protein (20 μg/lane) were separated via SDS-PAGE on a 12% gel. The separated proteins were transferred onto a polyvinylidene fluoride membrane (EMD Millipore). The membrane was blocked with 5% milk in PBS and 0.1% Triton X-100 at room temperature for 2 h. The membrane was incubated with primary antibodies against acetylated histone H4 lysine H4K5 (cat. no. ab114146; 1:1,000), acetylated H4K8 (cat. no. ab45166; 1:5,000) and H4 (cat. no. ab109463; 1:1,000; all Abcam) overnight at 4˚C. Following primary antibody incubation, the membrane was incubated with horseradish peroxidase-conjugated secondary antibodies (cat. no. SA00001-2; 1:2,000; ProteinTech Group, Inc.). Blots were visualized using an enhanced chemiluminescence (ECL) kit (cat. no. WP20005; Thermo Fisher Scientific, Inc.) and an ECL system (GE Healthcare). Protein expression was quantified using Quantity One software (version 2.4; Bio-Rad Laboratories, Inc.). Each experiment was performed in triplicate and H4 was used as the loading control.

Statistical analysis. Statistical analysis was performed using GraphPad Prism software (version 6.0; GraphPad Software, Inc.). Data are expressed as the mean ± standard deviation. Statistical differences between groups were analyzed using the one-way analysis of variance followed by the Tukey's post hoc test or student's t test. The basic information of the patients presented in Table I was analyzed using the χ^2 and Student-Newman-Keuls tests. P<0.05 was considered to indicate a statistically significant difference.

Results

Basic information of investigated subjects. A total of 180 puerperant were recruited and divided into three groups: FTL, TPROM and PPROM. Each group consisted on 60 patients. The mean age of the patients in the FTL, TPROM and PPROM groups was 27.5±3.67, 27.79±3.44 and 28.26±5.13 years, respectively. No difference in age distribution among the three groups was observed (P>0.05). There were no significant differences in one-child ratios (the number of babies born in the current study as the first baby of the mother over the number of all parturients included in the present study) x100%, and

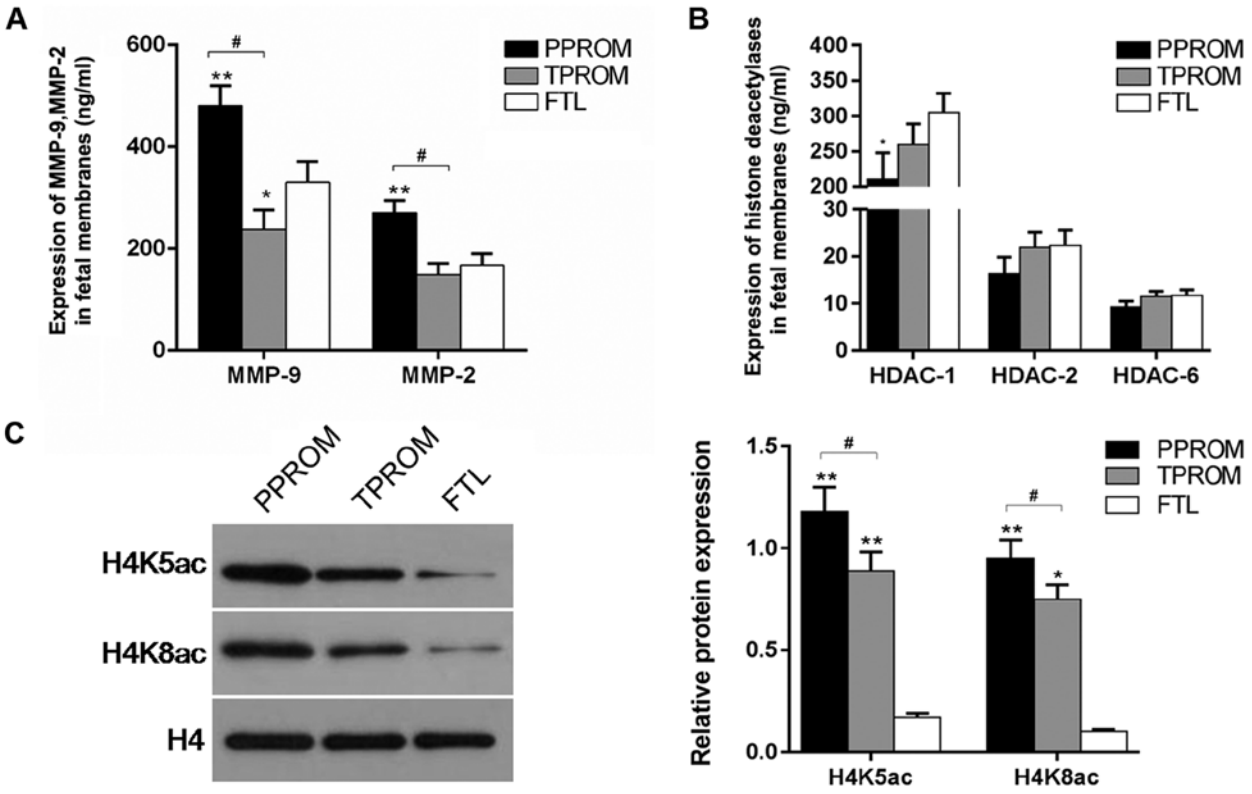

Figure 1. Expression levels of MMPs and histone acetylation-associated factors (H4K5ac and H4K8ac) in fetal membrane tissues. Fetal membranes were divided into three groups (FTL, TPROM and PPROM), and levels of (A) MMP2 and MMP9, and (B) HDAC1, HDAC2 and HDAC6 were detected using ELISA kits. (C) Western blotting was performed to assess the protein levels of H4K5ac and H4K8ac in FTL, TPROM and PPROM groups. H4 served as an internal control. Data are expressed as mean ± standard deviation from three independent experiments. *P<0.05 and **P<0.01 vs. the FTL group. $^{\#}$P<0.05 vs. the TPROM group. MMPs, matrix metalloproteinases; H4K, histone H4 lysine; FTL, full-term labor; PROM, premature rupture of membranes; TPROM, term-PROM; PPROM, pre-term-PROM; HDAC, histone deacetylase; ac, acetylated.

eosinophil and glucose levels among the three groups (P>0.05; Table I). However, significant differences in the body mass index, gestational age, time of membrane rupture to delivery, incidence of antepartum hemorrhage and serum albumin levels were observed between PPROM and FTL or between PPROM and TPROM (P<0.05; Table I). Moreover, neutrophil and albumin levels significantly affected premature rupture of membranes in the TPROM and PPROM groups, compared with the FTL group using SNK test (P<0.05). These results indicated that obesity, inflammation and infection may result in premature rupture of membranes.

Expression of MMPs, H4K5ac and H4K8ac in fetal membrane tissues. The current study investigated the associations between MMPs, H4K5ac and H4K8ac, and PROM. Compared with the FTL group, MMP9 concentration was significantly decreased in the TPROM group (P<0.05; Fig. 1A), and MMP2 and MMP9 concentrations significantly increased in the PPROM group (P<0.01; Fig. 1A). While the concentration of HDAC2 and HDAC6 appeared to be decreased in the PPROM group, no significant differences among the three groups were observed (P>0.05; Fig. 1B). HDAC1 concentration was decreased in the PPROM and TPROM groups compared with the FTL group, although only significantly so in the PPROM group (P<0.05; Fig. 1B). As presented in Fig. 1C, the levels of H4K5 and H4K8 acetylation in the PPROM and TPROM groups were significantly increased compared with the FTL group (all P<0.01 except H4K8 in TPROM, P<0.05). The levels of H4K5ac and H4K8ac were significantly increased in the PPROM group compared with the TPROM group (P<0.05; Fig. 1C).

Verification of HAEpi cells. To verify HAEpi cells, the epithelial cell marker CK19 was detected by immunofluorescence. As presented in Fig. 2, 98% of the cells were CK-19 positive, indicating that the cells were human amniotic epithelial cells.

Effects of droxinostat on cell viability and the levels of MMPs, H4K5ac and H4K8ac in HAEpi cells. The effect of increasing concentrations of droxinostat on the viability of HAEpi cells was investigated following 24, 48 and 72 h of incubation. The results revealed that as the droxinostat concentration increased the cell viability decreased (Fig. 3A). Droxinostat treated at 5 μM for 48 h was selected for subsequent experimentation as this concentration and incubation time significantly reduced the viability of HAEpi cells (P<0.05). Western blotting revealed that 5 μM droxinostat significantly enhanced the expression of H4K5ac and H4K8ac in HAEpi cells compared with the control (P<0.01; Fig. 3B). Compared with the control, 5 μM droxinostat significantly decreased the levels of MMP2 and MMP9 (P<0.01; Fig. 3C). Droxinostat did not significantly affect the levels of HDAC1 and HDAC2 compared with the control (P>0.05; Fig. 3D). However, 5 μM droxinostat

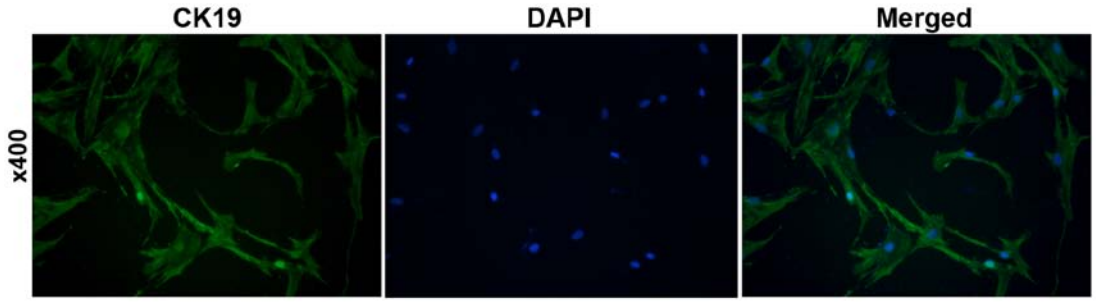

Figure 2. Verification of human amniotic epithelial cells using immunofluorescence. Images were taken with a fluorescence microscope (magnification, x400). CK19, cytokeratin 19.

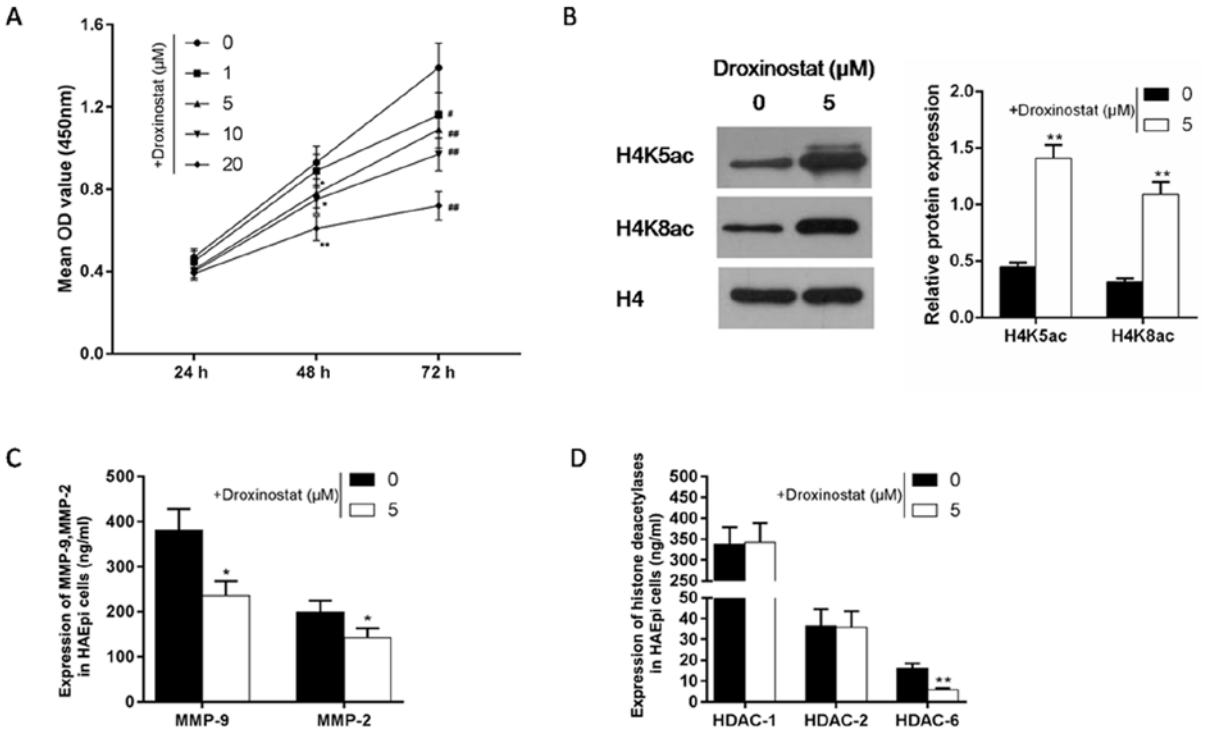

Figure 3. Effects of droxinostat on cell viability and levels of MMPs and histone acetylation related factors (H4K5ac and H4K8ac) in HAEpi cells. (A) A Cell Counting Kit-8 assay was performed to detect the effect of different concentrations (1, 5, 10 and 20 μM) of droxinostat on HAEpi cell viability following 24, 48 and 72 h. *P<0.05 and **P<0.01 vs. the control group at 48 h; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the control group at 72 h. (B) The effect of droxinostat on the levels of H4K5ac and H4K8ac in HAEpi cells was investigated using western blotting. H4 served as an internal control. The effects of droxinostat on (C) MMP2 and MMP9, and (D) HDAC1, HDAC2 and HDAC6 were detected by ELISA kits. Data are expressed as the mean ± standard deviation from three independent experiments *P<0.05 and **P<0.01 vs. the control group. MMPs, matrix metalloproteinases; H4K, histone H4 lysine; HAEpi, human amniotic epithelial; HDAC, histone deacetylase; ac, acetylated.

significantly decreased the level of HDAC6 compared with the control (P<0.01; Fig. 3D).

Effects of droxinostat on HAEpi cell invasion and migration. The effect of droxinostat on HAEpi cell invasion and migration was investigated using transwell assays. The results revealed that droxinostat significantly inhibited HAEpi cell invasion and migration compared with the control (P<0.05; Fig. 4).

Effects of chidamide on cell viability and the levels of MMPs, H4K5ac and H4K8ac in HAEpi cells. The effects of chidamide on cell viability, MMP concentrations, and protein expression levels of H4K5ac and H4K8ac in HAEpi cells were investigated in the current study. Increasing concentrations (0.1, 0.2, 0.5 and 1 μM) of chidamide were used to detect cell viability following 24, 48 and 72 h. The results revealed that chidamide decreased HAEpi cell viability in a manner similar to droxinostat (Fig. 5A). A concentration of 0.5 μM chidamide and incubated of 48 h was selected as this concentration/temperature significantly reduced HAEpi cell viability (P<0.05). As presented in Fig. 5B, chidamide significantly increased the levels of H4K5ac and H4K8ac compared with the control (P<0.01). The concentrations of MMP2 and MMP9 were significantly decreased in 0.5 μM chidamide-treated cells compared with controls (P<0.01; Fig. 5C). Compared with droxinostat, chidamide had different effects on the levels of HDAC1, HDAC2 and HDAC6 in HAEpi cells. The levels of HDAC1 and HDAC2 were significantly decreased following

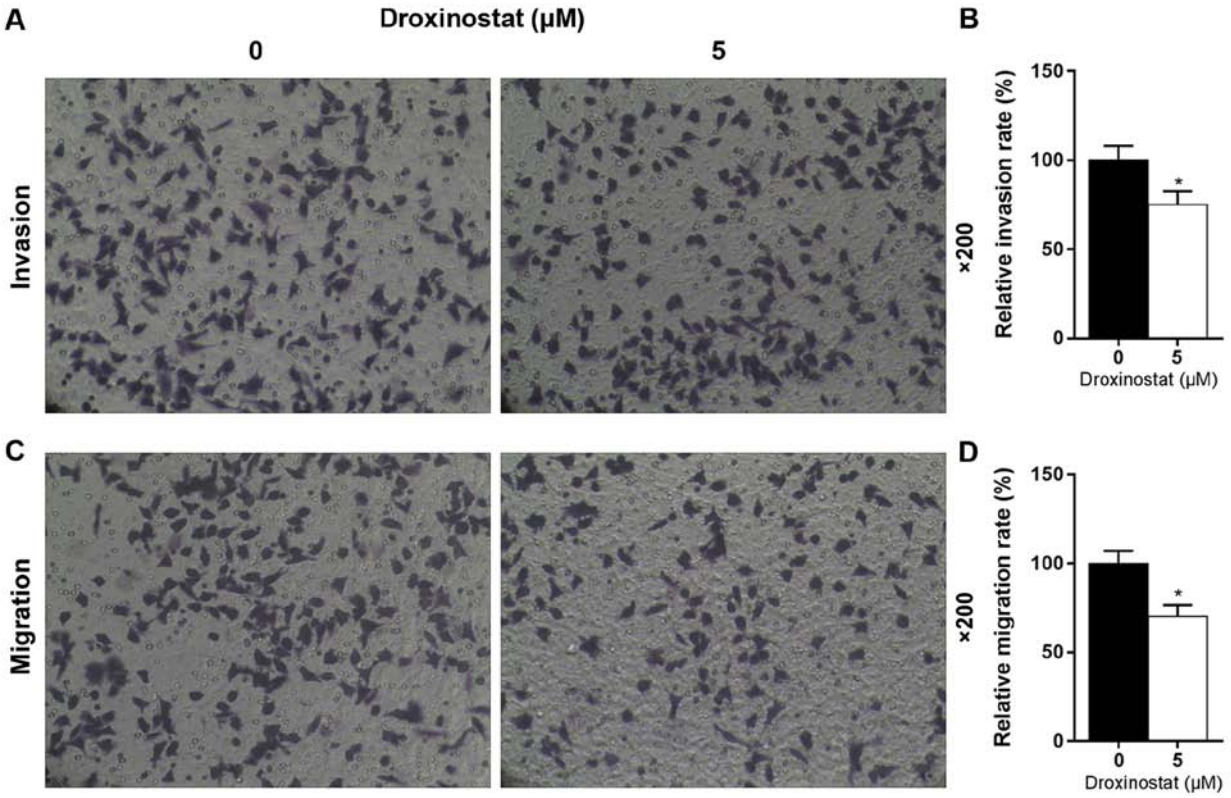

Figure 4. Effects of droxinostat on HAEpi cell invasion and migration assessed using transwell assays. (A) Cell invasion and (B) relative invasion rate of HAEpi cells. (C) Cell migration and (D) relative migration rate of HAEpi cells (magnification, x200). *P<0.05 vs. the control group. HAEpi, human amniotic epithelial.

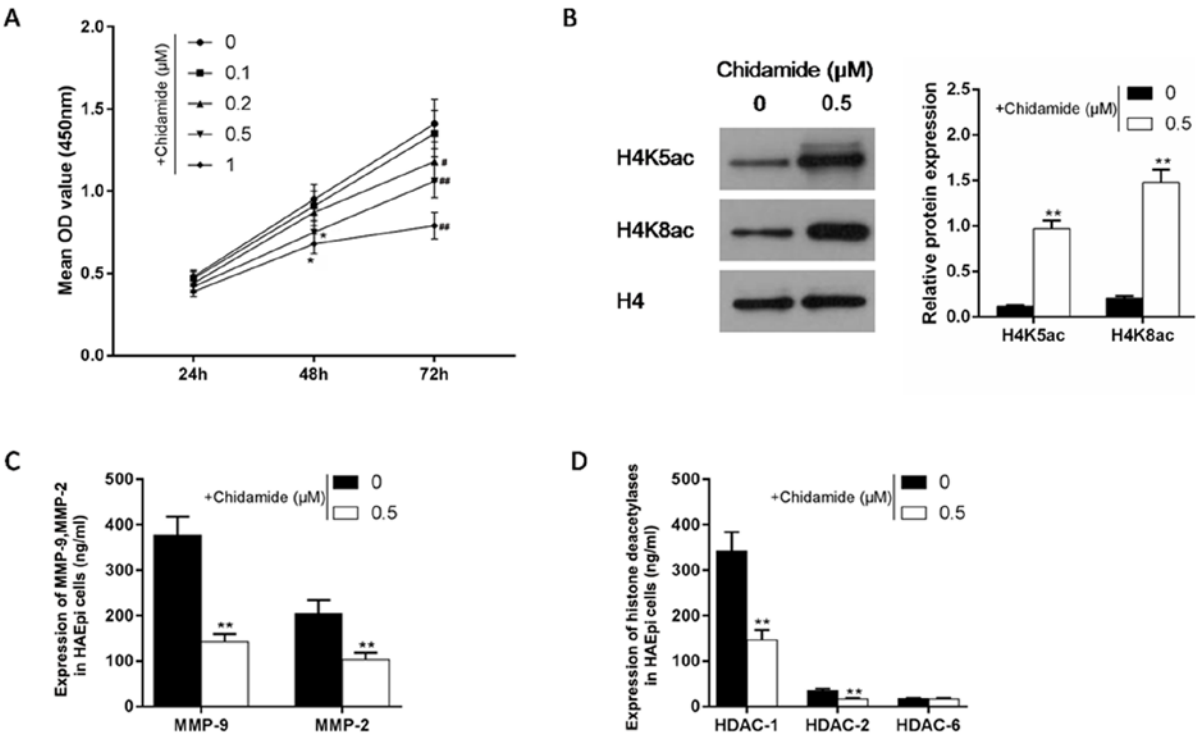

Figure 5. Effects of chidamide on cell viability and levels of MMPs and histone acetylation related factors (H4K5ac and H4K8ac) in HAEpi cells. (A) A Cell Counting Kit-8 assay was performed to detect the effect of different concentrations (0.1, 0.2, 0.5 and 1 μM) of chidamide on HAEpi cell viability following 24, 48 and 72 h. *P<0.05 vs. the control group at 48 h; #P<0.05 and ##P<0.01 vs. the control group at 72 h. (B) The effect of chidamide on the levels of H4K5ac and H4K8ac in HAEpi cells was investigated using western blotting. H4 served as an internal control. The effects of chidamide on (C) MMP2 and MMP9, and (D) HDAC1, HDAC2 and HDAC6 were detected by ELISA kits. Data are expressed as mean ± standard deviation from three independent experiments. *P<0.05 vs. the control group. MMPs, matrix metalloproteinases; H4K, histone H4 lysine; HAEpi, human amniotic epithelial; HDAC, histone deacetylase; ac, acetylated.

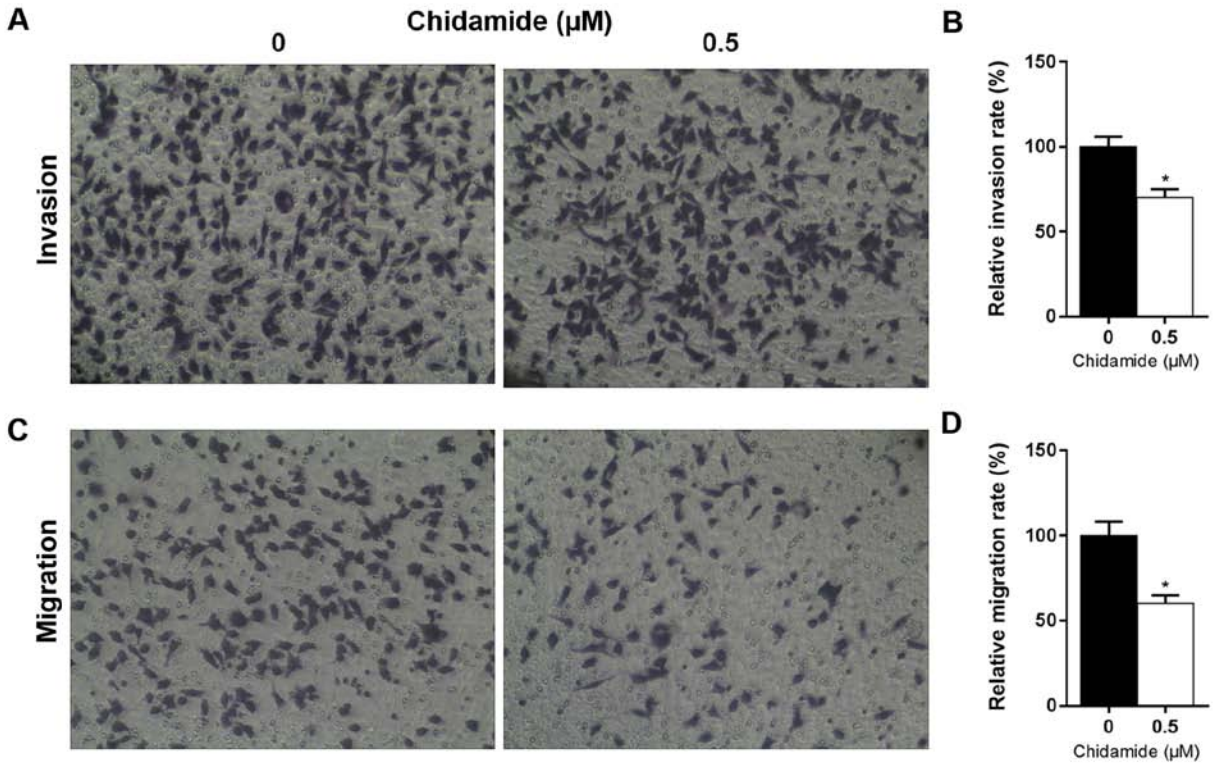

Figure 6. Effects of chidamide on HAEpi cell invasion and migration assessed using the transwell assay. (A) Cell invasion and (B) relative invasion rate of HAEpi cells. (C) Cell migration and (D) relative migration rate of HAEpi cells. *P<0.05 vs. the control group. HAEpi, human amniotic epithelial.

exposure to 0.5 μM chidamide compared with control (P<0.01; Fig. 5D). Chidamide did not significantly affect the level of HDAC6 compared with controls.

Effects of chidamide on HAEpi cell invasion and migration. Similar to the results obtained for 5 μM droxinostat, 0.5 μM chidamide significantly inhibited HAEpi cell invasion and migration compared with the control (P<0.05; Fig. 6).

Discussion

Premature rupture of fetal membranes may result in various adverse effects in the puerperant and fetus, including cerebral palsy, intellectual handicap and chronic lung disease (33). An increased understanding of the mechanisms underlying PROM and the development of novel interventions may prolong the gestational age of the parturient and improve the survival rate of newborns. Amnion and chorion exhibit collagen-dissolving reconstitution processes, which allow the membranes to overcome the effect of uterine growth (34). This process is closely associated with the activation of MMPs (34), which degrade the majority of the ECM components consisting of collagen, proteoglycan and glycoprotein (16). Therefore, MMPs may serve important roles in PROM (35).

The present study investigated 180 puerperant with FTL, TPROM or PPROM. The concentrations of MMP2 and MMP9 in fetal membranes were significantly different in PPROM and TPROM compared with FTL. Specifically, MMP concentrations were increased and decreased in the PPROM and TPROM groups, respectively, compared with the FTL group. Previous studies reported an increased expression of MMPs in PPROM (36-38). Certain inflammatory factors (including interleukin-1b and tumor necrosis factor-α) promote chorioamnionitis, which enhances the secretion of MMPs, degrades ECM components of the fetal membrane, decidua and cervix, and induces PROM and premature delivery (36-40). In the current study, lower concentrations of MMP2 and MMP9 in the TPROM groups suggested that other MMPs, for example MMP7, may serve a leading role in TPROM and that low levels of MMP2 are expressed at term (41,42).

The present study demonstrated that while HDAC1 expression was decreased in the PPROM and TPROM groups compared with the FTL group, the expression of acetylated histones increased. Histone modification serves an important role in the development of diseases such as malignant neoplasms, nephropathy, respiratory diseases, blood system diseases and reproductive diseases (21,22,43). Additionally, in chronic obstructive pulmonary disease, high histone H4 acetylation may cause excessive release of inflammatory factors, inducing an inflammatory reaction and accelerating the progression of the disease (44). A total of 18 mammalian HDACs have been identified and may be divided into four categories as follows: Class I (HDAC1-3 and HDAC8), class II (IIa, HDAC4, HDAC5, HDAC7 and HDAC9; IIb, HDAC6 and HDAC10), class III (sirtuin 1-7) and class IV (HDAC11) (45,46). HDAC1 is expressed in human endometrium and is closely associated with embryonic development (47).

The present study investigated the effects of histone acetylation on regulating the expression levels of MMPs in PROM by exposing HAEpi cells to histone deacetylase inhibitors *in vitro*. The results revealed that different concentrations of the histone deacetylase inhibitors droxinostat and chidamide

significantly inhibited cell viability compared with controls. Droxinostat and chidamide are widely used antitumor agents (48-51) and reduced HAEpi cell viability, invasion and migration in the current study, potentially by decreasing cell growth and increasing apoptosis (52,53). Furthermore, the present study revealed that droxinostat and chidamide significantly promoted the levels of H4K5 and H4K8 acetylation, and inhibited the secretion of MMP2 and MMP9, similar to what was observed in the TPROM group. Moreover, 5 μM droxinostat significantly decreased the expression level of HDAC6, and 0.5 μM chidamide significantly decreased the expression levels of HDAC1 and HDAC2 in HAEpi cells. The aforementioned results were in accordance with previous studies, which suggested that droxinostat selectively decreased HDAC3, HDAC6 and HDAC8 expression levels and promoted histone acetylation, while chidamide selectively decreased the expression levels of HDAC1, HDAC2, HDAC3 and HDAC10 (54,55). Therefore, future studies investigating different histone deacetylase inhibitors are required to demonstrate that histone deacetylase inhibitors decrease the expression of HDACs, and induce acetylation of H4K5 and H4K8. In the current study, exposure to droxinostat and chidamide increased histone H4 hyperacetylation in HAEpi cells, and an accompanying decrease in cell viability, and cell invasion and migration was observed. Furthermore, droxinostat and chidamide decreased the expression levels of MMP2 and MMP9, suggesting that histone H4 hyperacetylation may suppress MMP2 and MMP9 in a manner similar to that observed in TPROM.

The current study had a number of limitations. Western blotting was not performed to detect MMP and HDAC proteins. Additionally, *in vitro* experiments were performed using HAEpi and not PROM cells. Further investigations to validate the results obtained in the current study are required.

In conclusion, the present study demonstrated that high expression levels of MMP2 and MMP9 were observed in PPROM. Additionally, *in vitro* experiments suggested that the effect of histone H4 hyperacetylation on inhibiting MMP2 and MMP9 activities in HAEpi cell was similar to that observed in TPROM. The results obtained in the current study may guide clinical treatment for PROM.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZS, FF and WH conceived and designed the study. LY, LZ and JY acquired the data and performed the data analysis and interpretation. ZS drafted the article and critically revised it for important intellectual content. All authors gave final approval for the manuscript to be published.

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of the Maternal and Child Health Hospital of Tangshan (Tangshan, China) and written informed consent was obtained from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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