CEOG Clinical and Experimental Obstetrics & Gynecology

Decreased levels of UBE2Q1 and CHIP in the placentas of infection related preterm birth

Qingyan Cao[1*], Jing Zhang[1*], Li Zhang[1], Daijuan Chen[1,2], Bing Peng[1]

[1]Department of Obstetrics and Gynaecology, West China Second University Hospital, Sichuan University, Chengdu
[2]West China School of Medicine, Sichuan University Chengdu (China)

Summary

Objective: To explore the role of ubiquitin proteasome system in the pathogenesis of infection related preterm birth. *Materials and Methods:* Thirteen women with spontaneous preterm delivery and 13 controls were included in present case-controlled study. The mRNA and protein levels of Ub-conjugating enzyme E2Q1 (UBE2Q1) and Carboxyl-terminus of Hsc70 interacting protein (CHIP) in the placenta were measured by immunohistochemistry, real-time RT-PCR, and Western blotting methods. Statistical significance ($p < 0.05$) was determined using student's *t*-test. *Results:* The mRNA levels of UBE2Q1 (0.48±0.05 *vs.* 0.67±0.07, $p = 0.047$) and CHIP (1.59±0.23 *vs.* 5.62±1.00, $p = 0.002$) in the placentas of preterm delivery were significant lower than those of term delivery. The protein levels of UBE2Q1 (0.64±0.09 *vs.* 1.49±0.22, $p < 0.001$) and CHIP (0.76±0.08 *vs.* 1.33±0.23, $p = 0.001$) in the placentas of preterm delivery were also significant lower than those of term delivery. *Conclusions:* Decreased mRNA and protein expression of UBE2Q1 and CHIP were both found in the syncytiotrophoblast and cytotrophoblast of placenta in preterm birth patients, which was speculated to play a role in the pathogenesis of infection related preterm birth.

Key words: Ubiquitin proteasome system; UBE2Q1; CHIP; Infection; Preterm birth.

Introduction

Preterm birth is one of the most significant healthcare issues facing perinatal medicine. It is also the leading cause of neonatal deaths and associated with various infant disorders, such as lifelong neurologic handicaps, acute respiratory illness, and other chronic diseases that can extend into adulthood. The estimated prevalence of preterm birth ranges from 5% to 18% worldwide [1], levying a considerable cost burden to the healthcare system, and emotional and financial strain to the families affected. Thus, it is vital to understand the molecular and cellular mechanisms that govern birth to prevent the serious clinical sequelae associated with preterm birth.

Among the many causes of preterm birth, inflammation/infection is responsible for the majority of early preterm births [2]. The underlying infection, such as those caused by chlamydia trachomatis and ureaplasma urealyticum, is considered to be one of the etiologies of preterm delivery. Previous studies have shown that reduction in CD1d surface expression through ubiquitin proteasome system (UPS) mediated protein degradation may help chlamydia trachomatis evade detection by innate immune cells. Misregulation of UPS assists chlamydia trachomatis to evade immune clearance and promote microorganism persistence [3]. UPS is speculated to be involved in the pathogenesis of infection related preterm birth. However, no study explored the role of UPS in the process of infection-related preterm birth.

Ub-conjugating enzyme E2Q1 (UBE2Q1) is a member UPS family and plays critical roles in female fertility and embryo implantation process. Reduction in UE2Q1 activities have been associated with different reproductive defects in females. UBE2Q1 expression is not detectable in the uterus of non-pregnant females but strongly up-regulated during pregnancy [4]. Carboxyl-terminus of Hsc70 interacting protein (CHIP), originally identified as a co-chaperone of Hsc70, has both a U-box domain and a tetratricopeptide repeat (TPR) motif. The U-box domain possesses E3 ubiquitin ligase activity, while the TPR motif associates with Hsc70 and Hsp90 [5]. It is found to mediate the degradation of estrogen receptor and thus may regulate female hormonal responses [6, 7]. In the present study, the authors examined the mRNA and the protein levels of UBE2Q1 and CHIP, in the placenta to explore their potential roles in the infection-related preterm birth.

Materials and Methods

This study was approved by the Ethics Committee of West China Second University Hospital. All women were informed and signed the consents to donate their placenta for scientific research use. Placenta tissues were obtained from 13 women with spontaneous preterm delivery who delivered vaginally between 28+0

[*]Contributed equally.

Revised manuscript accepted for publication September 14, 2017

and 36+6 weeks of gestation. All the patients has clinical manifestation of infection such as fever, increased white blood cell or C-reaction protein. Normal term placenta tissues (>37+n weeks gestation) were also collected from 13 women with normal vaginal delivery as control. Other complications of pregnancy, such as pre-eclampsia, asthma, gestational diabetes mellitus, multiple pregnancies, and fetuses with chromosomal abnormalities, were excluded from this analysis.

Placenta samples were fixed with formaldehyde for immunohistochemistry, or frozen in liquid nitrogen within one hour of excision and stored at -70°C for future RNA and protein analysis. All placentas collected from preterm deliveries were swabbed for microbiological culture investigations and histopathological examination to confirm chorioamnionitis.

To determine the subcellular distributions of UBE2Q1 and CHIP in placenta, immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections with the biotin-streptavidin staining procedure). Sections were dewaxed in xylene twice and rehydrated through a gradient of ethanol. Slides were boiled in citrate buffer (10 mM citrate sodium, 10 mM citric acid, pH 6.0) at 98°C for 15 minutes to retrieve antigen, cooled at RT for 25 minutes, washed with PBS three times, and incubated with 3% hydrogen peroxide for 15 minutes to quench endogenous peroxidase activity. Then the slides were incubated with UBE2Q1 antibodies, or CHIP antibody, both at 1:100 dilution and 37°C for one hour. After washing in PBS, the slides were incubated with HRP-conjugated secondary antibody and developed with DAB. For the negative control, non-specific matched IgG was used to replace the primary antibody. Slides were counter-stained with hematoxylin, visualized with a light microscope and photographed with a color video camera. Slides were reviewed by well-experienced pathologists in parallel.

Total RNA was extracted from placenta samples using TRIzol regent following the manufacturer's instructions. The integrity of RNA was confirmed by agarose gel electrophoresis. Reverse transcription was performed with 5 μl of total RNA pretreated with RNase-free DNase and random primer using a cDNA synthesis kit, and incubated on the real-time PCR system for 60 minutes at 42°C and 10 minutes at 72°C. Real-time PCR was performed using 5 μl cDNA, Taq DNA polymerase, and dNTP on a real-time PCR system. The PCR program was initiated at 94°C for 2 minutes, followed by 45 thermal cycles of 20 seconds at 94°C, 20 seconds at 55°C, and 30 seconds at 60°C. The expression of the UBE2Q1 and CHIP were normalized to the expression of ACTB and expressed as fold change relative to negative controls. The following primers were used: UBE2Q1 (Forward: GTCCGCATCCACTGCAACAT, Reverse: CTCCAAGACAGCAGCCAAGT), CHIP (Forward: GAAGAGTGCCAGCGAAACCA, Reverse: CGCATCAGCTCAAAGCTGAT), ACTB (Forward: GAAGATCAAGATCATTGCTCCT, Reverse: TACTCCTGCTTGCTGATCCACA). A melting curve for primer validation and a template standard curve were performed to show template-independent amplification results. Data were analyzed with the comparative delta Ct method. In addition, amplimers were systematically controlled by agarose gel electrophoresis and sequencing.

Placenta tissues were homogenized in a tissue homogenization buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 0.2% Triton X-100, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 1× complete protease inhibitor cocktail). The lysates were then centrifuged for 15 minutes at 12,000 g at 4°C. The protein concentration in the supernatant was determined by the Bradford assay. The protein samples were supplemented with SDS sample buffer, boiled for 6 minutes, and separated by SDS-polyacrylamide gel electrophoresis. Proteins were then transferred electrophoretically onto a nitrocellulose blotting membrane. After being blocked with 5% fat free milk for one hour at RT, the membrane was incubated with UBE2Q1 (1:300), or CHIP (1:300) antibody at 4°C overnight. β-actin (1:300) was detected as the control to normalize the amounts of protein. Blots were washed three times in PBS-Tween (PBS-T) and incubated with secondary antibodies (1:20000) at 37°C for one hour. A chemiluminescent substrate were used for detecting the bands on membranes. Light emission was captured by exposing the membrane to X-ray films. The levels of UBE2Q1 and CHIP proteins were estimated by densitometric quantitation of the intensity of the signal from the bands in relation to that from the β-actin band using Quantity One Analyzer software (V4.4.0.36).

This study was approved by the Ethics Committee of West China Second University Hospital. All women were informed and signed the consents to donate their placenta for scientific research use.

Data are expressed as mean±SD. Statistical significance ($p < 0.05$) was determined using student's *t*-test. Analyses were performed using the Statistical Package for Social Science (SPSS16.0).

Results

The average gestational age of preterm delivery was 34.02±1.60 weeks. All the patients in the preterm delivery group had clinical infection signs such as fever, elevated white blood cell or C-reaction protein. Pathological examination of the placenta of preterm delivery group showed seven cases of mild chorioamnionitis, two cases of moderate chorioamnionitis, and two cases of severe chorioamnionitis. All the premature infants were transferred to the NICU and all were discharged after carefully treatment.

UBE2Q1 and CHIP proteins were both found to be expressed in the cytoplasm of syncytiotrophoblast and cytotrophoblast of third trimester placenta by immunohistochemistry analysis. Results were shown in Figure 1.

Real-time PCR revealed that the UBE2Q1 mRNA levels in the placentas of preterm delivery were significant lower than those of term delivery (0.48±0.05 *vs.* 0.67±0.07, $p = 0.047$). The CHIP mRNA levels in the placentas of preterm delivery were also lower than those of term delivery (1.59±0.23 *vs.* 5.62±1.00, $p = 0.002$). Detailed results are listed in Table 1.

Consistent with the mRNA results, Western blot analysis showed that the UBE2Q1 protein levels in the placentas of preterm delivery were significant lower than those of term delivery (0.64±0.09 *vs.* 1.49±0.22, $p < 0.001$). The protein levels of CHIP in placentas of preterm delivery were also significant lower than those of term delivery (0.76±0.08 *vs.* 1.33±0.23, $p = 0.001$). The results are illustrated in Figure 2.

Discussion

UPS is one of the major pathways for intracellular protein degradation. It involves a three-step reaction to achieve a covalent attachment of ubiquitin molecules to lysine residues of the target protein [8]. UPS has been implicated

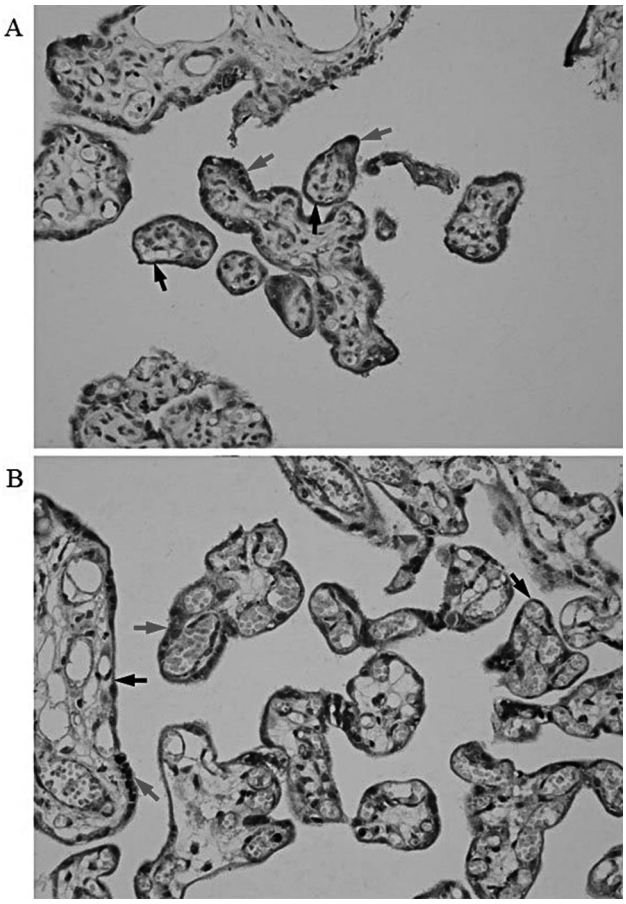

Figure 1. — UBE2Q1 and CHIP protein expression in third trimester placenta. UBE2Q1(A) and CHIP(B) are both expressed in cytoplasm and in cell membrane of the cytotrophoblast (black arrow) and syncytiotrophoblast (red arrow) respectively. (Magnification ×400).

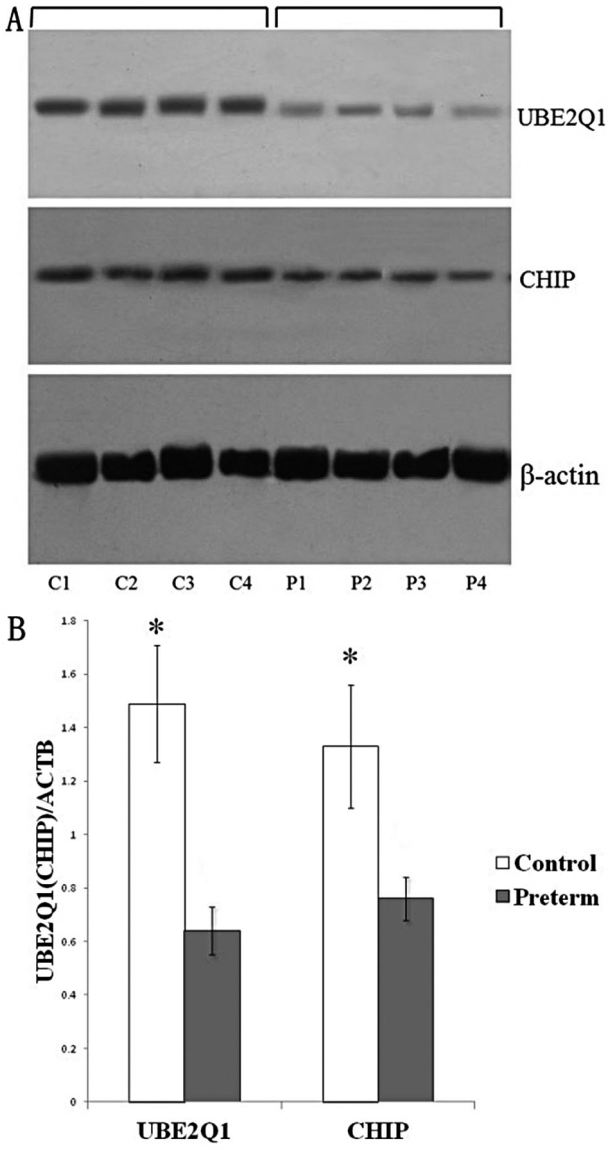

Figure 2. — Protein expression pattern of UBE2Q1 and CHIP. A, UBE2Q1 and CHIP protein expression in term (C1-C4) and preterm (P1-P4) placental villous tissues are determined by Western blot analysis. β-actin is used as a loading control. (B) Quantitative analysis of data shown in (A) of the intensity of UBE2Q1 ($p < 0.001$) and CHIP ($p = 0.001$).
*$p < 0.05$ between preterm and control group.

in protein degradation, transcriptional activation, signal transduction, and cell growth and differentiation [9]. Defects in UPS have been implicated in cancer development and progression, neurodegenerative disorders, pathogenic infections, preimplantation embryo development, and fetal implantation. In this study, the authors confirmed the protein expression of two important members of UPS, UBE2Q1 and CHIP, in the cytoplasm of syncytiotrophoblast and cytotrophoblast of third trimester placenta. They also found a dramatic decrease of UBE2Q1 and CHIP mRNA and protein expression in the placentas of preterm birth, suggesting the possible relationship between UPS impairment in placenta and preterm delivery.

UPS is found to be involved in the process of reproduction, especially in the initiation of pregnancy, including embryo implantation and placenta formation. However, the functions of UPS in the third trimester of pregnancy or in the complication of pregnancy are not very clear. Previous studies focused on the role of UPS in the preeclampsia and intraunterine growth retardation (IUGR). The E3 ubiquitin ligase MULE has been found to play an important role in the pathology of pre-eclampsia and IUGR by regulating myeloid cell leukemia factor 1 and tumor suppressor p53 [10]. The relationship between UPS and preterm delivery has not been carefully studied.

UBE2Q1 play vital role in female fertility and embryo implantation process. It is not expressed in the uterus of

Table 1. — *The mRNA levels of UBE2Q1 and CHIP in the placentas of preterm or term delivery women.*

No.	UBE2Q1*		No.	CHIP**	
	Preterm	Control		Preterm	Control
1	0.57	1.00	1	1.00	8.00
2	0.71	0.70	2	0.87	9.19
3	0.35	0.41	3	0.25	11.31
4	0.20	0.57	4	2.82	9.19
5	0.50	0.57	5	1.74	9.19
6	0.28	1.05	6	1.41	2.82
7	0.70	0.51	7	0.72	1.74
8	0.51	1.23	8	1.23	1.86
9	0.71	0.50	9	1.05	2.05
10	0.71	0.61	10	2.83	8.00
11	0.25	0.71	11	2.46	2.00
12	0.43	0.52	12	2.29	5.65
13	0.31	0.35	13	2.00	2.14
Mean±SD	0.48±0.05	0.67±0.07	Mean±SD	1.59±0.23	5.62±1.00

* *UBE2Q1: Ub-conjugating enzyme E2Q1.*

***CHIP: Carboxyl-terminus of Hsc70 interacting protein.*

non-pregnant females, but its expression was up-regulated in the uterus during pregnancy. It is involved in some female reproductive defects of female fertility, including increased embryonic lethality and decreased implantation capacity of homozygous mutant embryos [4]. However, potential roles of UBE2Q1 in ubiquitination and reproduction need further investigation. Decreased mRNA and protein levels of UBE2Q1 were found in the placenta of preterm delivery patients in the present study. The present authors speculated that it might facilitate the ubiquitination of p53 and lead to subsequent p53 degradation. The reduced level of UBE2Q1 in preterm delivery placenta might attenuate the turnover of p53 in trophoblast cell, leading to excessive p53 accumulation [11]. However, deletion of uterine *Trp53* was found to induce preterm birth through a COX2/PGF synthase/PGF2α pathway [12]. The results are contradictory, and further study is needed to identify the target proteins and detailed mechanisms between UBE2Q1 and preterm delivery.

Autophagy enables the cell to sequester deleterious microorganisms within autophagosome that could fuse with lysomes to degrade the pathogens. It represents a key barrier to intracellular pathogens growth. Autophagic capture of microorganisms occurs via an ubiquitin-dependent process by surrounding the microorganisms with a coat of polyubiquitin. Ubiquitination was found to play a key role in marking cytosol-exposed bacteria for autophagy. LRSAM1 was found to be both a bacterial recognition protein and an ubiquitin E3 ligase that participates in the autophagic pathway and defends the cytoplasm from invasive pathogens [13]. Another ubiquitin E3 ligase CHIP has been found to be involved in both the protease degradation process and the selective autophagy pathway by marking the target proteins [14]. In present study, the authors found that the mRNA and protein expression of CHIP was decreased in the placentas of preterm delivery, and they speculated that CHIP might be the E3 ligase involved in ubiquitin-dependent anti-pathogen autophagy pathway in the trophoblast cell, which might play vital role in preventing preterm delivery.

Conclusions

To the best of the present authors' knowledge, this is the first study to show that UPS might be involved in the process of preterm labor. Decreased levels of UBE2Q1 and CHIP are both found in the syncytiotrophoblast and cytotrophoblast of placenta in preterm birth patients. However, the limitation of the present study is not elucidating the detailed mechanisms of UBE2Q1 and CHIP in the pathogenesis of preterm delivery which need further research.

Acknowledgement

This study was supported for sample collection and tests performing by the Applied Basic Research Programs of the Scientific and Technological office of Sichuan Province (2015JY0134).

References

[1] Blencowe H., Cousens S., Oestergaard M.Z., Chou D., Moller A.B., Narwal R., *et al.*: "National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet,* 2012, 379, 2162.

[2] Goldenberg R.L., Culhane J.F., Iams J.D., Romero R.: "Epidemiology and causes of preterm birth". *Lancet,* 2008, *371*, 75.

[3] Kawana K., Quayle A.J., Ficarra M., Ibana J.A., Shen L., Kawana Y., *et al.*: "CD1d degradation in Chlamydia trachomatis-infected epithelial cells is the result of both cellular and chlamydial proteasomal activity". *J. Biol. Chem.,* 2007, *282*, 7368.

[4] Grzmil P., Altmann M.E., Adham I.M., Engel U., Jarry H., Schweyer S., *et al.*: "Embryo implantation failure and other reproductive defects in Ube2q1-deficient female mice". *Reproduction,* 2013, *145*, 45.

[5] Murata S., Chiba T., Tanaka K.: "CHIP: a quality-control E3 ligase collaborating with molecular chaperones". *Int. J. Biochem. Cell. Biol.,* 2003, *35*, 572.

[6] Zhang Q.G., Han D., Wang R.M., Dong Y., Yang F., Vadlamudi R.K., *et al.*: "C terminus of Hsc70-interacting protein (CHIP)-mediated degradation of hippocampal estrogen receptor-alpha and the critical period hypothesis of estrogen neuroprotection". *Proc. Natl. Acad. Sci. U S A,* 2011, 108, E617.

[7] Fan M., Park A., Nephew K.P.: "CHIP (carboxyl terminus of Hs 70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor-alpha". *Mol Endocrinol.,* 2005, *19*, 2901.

[8] Pickart C.M.: "Back to the future with ubiquitin". *Cell*, 2004, *116*, 181.

[9] Johnson D.: "The ubiquitin-proteasome system: opportunities for therapeutic intervention in solid tumors". *Endocr. Relat. Cancer,* 2015; *22*, T1.

[10] Rolfo A., Garcia J., Todros T., Post M., Caniggia I.: "The double life

of MULE in preeclamptic and IUGR placentae". *Cell Death Dis.,* 2012; *3*, e305.

[11] Wan C., Chen J., Hu B., Zou H., Li A., Guo A., *et al.*: "Downregulation of UBE2Q1 is associated with neuronal apoptosis in rat brain cortex following traumatic brain injury". *J. Neurosci. Res.,* 2014, *92*, 1.

[12] Hirota Y., Daikoku T., Tranguch S., Xie H., Bradshaw H.B., Dey S.K.: "Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice". *J. Clin. Invest.,* 2010, *120*, 803.

[13] Huett A., Heath R.J., Begun J., Sassi S.O., Baxt L.A., Vyas J.M., *et al.*: "The LRR and RING domain protein LRSAM1 is an E3 ligase crucial for ubiquitin-dependent autophagy of intracellular Salmonella Typhimurium". *Cell Host Microbe,* 2012, *12*, 778.

[14] Kraft C., Peter M., Hofmann K.: "Selective autophagy: ubiquitin-mediated recognition and beyond". *Nat. Cell Biol.,* 2010, *12*, 836.

Corresponding Author:
BING PENG, M.D.
Department of Obstetrics and Gynaecology
West China Second University Hospital
Sichuan University,
No.20, Section 3 Renmin Nanlu Road
Chengdu, 610041 (China)
e-mail: pengbin-a111@163.com