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Activation of Autophagy in Human Uterine Myometrium During Labor

Lele Wang, MD[1,2], Huiping Hu, MD[2], Abraham Nick Morse, MD, MBA[2], Xinjia Han, PhD[2], Junjie Bao, MsC[2], Jingying Yang, MD[2], Yunshan Chen, MD[2], and Huishu Liu, MD, PhD[2]

Abstract

Objective: The purpose of this study was to analyze the autophagy of the human uterine myometrium during the labor. **Methods:** We collected uterine myometrium strips from term, singleton, nulliparous healthy women undergoing cesarean delivery before labor (nonlabor group, n = 10) or during normal labor (in-labor group, n = 10) without rupturing of membrane. The indications for cesarean delivery were breech presentation or maternal request. Transmission electron microscopy was used to observe autophagosomes. Reverse transcriptase polymerase chain reaction, immunofluorescence, and Western blot were used to quantify the messenger RNA (mRNA) and protein level of the autophagy markers LC3B, P62, and Beclin-1 in the uterine muscle strips. **Results:** There were no differences between both groups in maternal age, body mass index, gestational week, neonatal weight, operative bleeding, and postpartum bleeding. Transmission electron micrographs showed that autophagosomes existed in myometrial tissue in both groups. There were more autophagosomes in the in-labor group than in the nonlabor group, and the difference had significance. The in-labor group had significantly greater LC3B mRNA expression but significantly lower P62 mRNA expression compared with the nonlabor group. Semiquantitative immunofluorescence in uterine myometrial cells in the in-labor group showed increased LC3B puncta formation and greater Beclin-1 expression but reduced P62 puncta formation compared with the nonlabor group. The ratio of LC3BII/I proteins was significantly higher, but P62 protein was significantly lower in the in-labor group compared with the nonlabor group. The Beclin-1 mRNA and protein expressions were not significantly different between the 2 groups. **Conclusion:** Autophagy was activated in human uterine myometrium during labor and might play an important role in maintaining uterine contraction function.

Keywords

autophagy, uterine myometrium, labor, hypoxia

Introduction

Successful birth is a result of complex events that are tightly regulated by a variety of mechanisms and mediators of the endocrine, nervous, and immune systems.[1] Uterine contractions are central to the delivery process. To achieve unassisted vaginal delivery, well-synchronized myometrial contractions need to become progressively stronger.[1,2] Failure to augment uterine contractility significantly increases the chance of cesarean delivery[3-5] and increases the risk of morbidity and mortality for the mother and baby.[3,4] Myometrial activation is a multifaceted process that involves alterations in receptors, changes in ion channel composition, and increases in gap junctions.[1,2] These processes have been shown to be regulated by inflammatory mediators, estrogen, progesterone, prostaglandin, corticotropin-releasing hormone, and various cytokines.[1,2,4] However, the underlying mechanisms for maintaining effective contractions during labor are still not well understood.

A number of studies have confirmed that contractions compress uterine vessels,[6-8] resulting in repetitive hypoxia-related metabolic stress of the myometrium.[9] Other organs, such as the brain and heart, are very sensitive to hypoxic injury.[10,11] However, in normal labor, the uterus endures repetitive periods of hypoxia lasting up to 20 hours while maintaining its normal contractile function.[12] These observations raise some important questions. For example, how does the uterus avoid hypoxic

[1] Department of Obstetrics, First Affiliated Hospital of Jinan University, Guangzhou, China
[2] Department of Obstetrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China

Corresponding Author:
Huishu Liu, Department of Obstetrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 9 Jinsui Road, Guangzhou, China.
Email: liuhuishu@gwcmc.org

injury? and how does uterine muscle maintain energy output and consume the hypoxic metabolic products that are produced during labor?

One possible mechanism involves autophagy. In response to environmental stresses such as hypoxia, autophagy is activated to sequester and digest cytoplasmic components such as damaged organelles, protein aggregates, and invading organisms through a lysosomal pathway. Autophagy may function as a protective pathway during ischemia in myocardial cells by degrading damaged mitochondria.[13,14] In other organ systems, autophagy appears to be important for bioenergetic management and cell survival.[14-16]

Here, we focused on the autophagy status in human uterine myometrium during the labor in which it suffered the repetitive hypoxia-related metabolic stress. Specifically, myometrial biopsies were collected from women at term undergoing cesarean delivery during labor or before the onset of labor. Autophagy-associated differences between the 2 groups were identified through electron microscopy (EM), immunofluorescence, reverse transcriptase polymerase chain reaction (RT-PCR), and Western blot.

Materials and Methods

Study Populations and Tissue Collection

Myometrial biopsies were obtained from term, singleton, nulliparous women undergoing cesarean delivery during or before labor in Guangzhou Women and Children's Medical Center.

The indication for the cesarean delivery was breech presentation or maternal request without any other medical reasons. All the patients in in-labor group were at the latent phase of the spontaneous labor when the cervix dilated from 0 to 3 cm without rupturing of membranes. Myometrial samples were collected immediately after delivery by elective cesarean delivery, and the biopsy was obtained from the upper edge of lower segment uterine incision randomly. Women with any of the following were excluded: (1) medical complications including hypertension, eclampsia, cholestasis, gestational diabetes, and other diseases; (2) abnormal labor including uterine atony or prolonged labor; (3) fetal abnormality including fetal distress, macrosomia, or malformation; and (4) placenta abnormality including placental abruption, placenta previa, or infection. The tissue specimens were snap-frozen in liquid nitrogen and stored at −80°C. As needed, they were embedded in paraffin for immunostaining or in EM fixative for transmission electron microscopy (TEM).

The study was approved by the ethics committee of Guangzhou Women and Children medical center (No. 2018041701). All women provided written informed consent.

Transmission Electron Microscopy

For autophagosome observation, TEM was used as previously described.[13,17] Myometrium were cut into 1-mm pieces immediately after obtaining from the upper edge of lower segment uterine incision randomly and placed into EM fixative (2% glutaraldehyde, 0.1 M sodium cacodylate) at 4°C. The specimens were washed with double distilled H_2O for 30 minutes 3 times and were dehydrated in 50% to 100% and then pure propylene oxide. The samples were embedded in EPON at room temperature for 12 hours, and the resin was polymerized in an oven at 55°C for 1 day. Next, 80-nm sections were cut and coated with lead citrate and uranyl acetate. Fields were examined using a transmission electron microscope (JEM-1400, JEOL, Tokyo, Japan). There were a total of 20 samples used for TEM imaging, half in-labor group and half nonlabor group. Ten visions of each section were selected randomly in magnified fields (500 μm). Autophagosomes were quantified by 2 independent observers (L.W. and X.H.) for each specimen. Both observers were blinded as to which group was examined.

Immunofluorescence

Immunofluorescence was used to visualize the location and density of LC3B, P62, and Beclin-1. The studies were carried out as previously described,[18] using 1:200 rabbit polyclonal anti-LC3B antibody (catalog #: L7543; Sigma, St Louis, Missouri), 1:50 rabbit polyclonal anti-P62 antibody (catalog #: ab91526; Abcam, Cambridge, United Kingdom), and 1:200 rabbit polyclonal anti-Beclin-1 antibody (catalog #: ab62557; Abcam). The secondary antibody was 1:300 goat anti-rabbit immunoglobulin G (H&L) Cy3 (catalog #: GB21303; Servicebio, Wuhan, China). Nuclei were subsequently counterstained with DAPI (catalog #: G1012; Servicebio). Under UV light, the nuclei appear blue, and positive antibody expression is labeled red. Immunoreactivity of autophagy markers was evaluated using 40 visions from each group (4 random visions per section under ×900 magnification). Using the same light exposure time and magnification, images from the myometrium area were taken. Image J (V1.31) was used to measure the fluorescence density. The mean ratio was determined by the fluorescence intensity and analyzed between groups. Fluorescence images were viewed and captured using Nikon Eclipse C1 microscope (Nikon, Tokyo, Japan).

Reverse Transcriptase Polymerase Chain Reaction

Total RNA was extracted from uterine myometrium using RNAiso Plus (catalog # 9109; Takara, Dalian, China) extraction, and the RNA concentration was measured by ultraviolet spectroscopy (Q6000UV; Quawell Technology, San Jose, California). Total RNA (1.5 μg) was used in the Bestar quantitative Polymerase Chain Reaction Reverse Transcription kit (catalog # 2220; DBI, Ludwigshafen, Germany) under the following conditions: initial denaturation at 95°C for 2 minutes, followed by 40 cycles (94°C for 20 seconds, 58°C for 20 seconds, 72°C for 20 seconds) and resolution melting (94°C for 30 seconds, 65°C for 30 seconds, 94°C for 30 seconds) using Bestar qPCR MasterMix (catalog # 2043; DBI). Experiments were performed in triplicate samples and repeated 3 times. The primer sequences for each gene are shown in Table 1.

Table 1. The Sequences of Primers of Human LC3B, P62, Beclin-1, and GAPDH.

Gene	Sequence 5′→3′	Size of PCR Product
LC3B	Forward primer: AGCATCCAACCAAAATCCCG Reverse primer: AGGAAGAAGGCCTGATTAGC	166 bp
P62	Forward primer: GCACCCCAATGTGATCTGC Reverse primer: CGCTACACAAGTCGTAGTCTGG	92 bp
Beclin-1	Forward primer: GGTGTCTCTCGCAGATTCATC Reverse primer: TCAGTCTTCGGCTGAGGTTCT	121 bp
GAPDH	Forward primer: TGTTCGTCATGGGTGTGAAC Reverse primer: ATGGCATGGACTGTGGTCAT	154 bp

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PCR, polymerase chain reaction.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control for gene expression analysis. Relative gene expression was determined as the ratio of the target gene to the internal reference gene expression (GAPDH) based on Ct values.

Western Blot

Protein was extracted from frozen myometrium with RIPA lysis buffer (G2002; Servicebio). Protein concentration was measured using a bicinchoninic acid protein assay kit (G2026; Servicebio), according to the manufacturer's instructions. Western blot protein samples were loaded in sodium dodecyl sulfate–polyacrylamide gels, resolved by electrophoresis, and transferred onto polyvinylidene difluoride membranes (IPVH00010; Merck Millipore, Billerica, Massachusetts). Protein levels were quantified by a ChemiDoc XRS+ (catalog # 23227; Thermo, Rockford, Illinois). The GAPDH was used as loading control. Western blotting antibodies used were 1:1000 anti-LC3B (catalog #: L7543; Sigma), 1:1000 P62 (catalog #: ab91526; Abcam), 1:2000 Beclin-1 (catalog #: ab62557; Abcam) and 1:10000 GAPDH (catalog #: ab91526; Abcam). Every sample was replicated for 3 times.

Statistical Analysis

Data for continuous variables were presented as mean ± standard error of the mean. The statistical significance of the results was assessed by 1-way analysis of variance or Welch test if variances were not similar. All statistical analyses were performed using the SPSS 25.0 software package (SPSS, Chicago, Illinois), with P value less than .05 being considered significant.

Results

Clinical and demographic characteristics of the study participants are summarized in Table 2. A total of 20 nulliparous women with full-term pregnancy were enrolled in this study. Ten women were in nonlabor group and 10 were in the in-labor group. The demographic and obstetrical characteristics of all participants are shown in Table 2. Both groups were similar in maternal age, body mass index, gestational week, neonatal weight, operative, and postpartum bleeding.

Table 2. Clinical Characteristics of the Study Participants.

Parameters	Nonlabor Group (n = 10)	In-Labor Group (n = 10)	*P* Value
Age (years)	29.0 ± 3.6	29.9 ± 4.1	.62
Body mass index at the end of pregnancy	25.4 ± 2.7	26.7 ± 6.9	.30
Gestational week (week)	39.5 ± 0.7	40.0 ± 0.7	.11
Neonatal weight (grams)	3205 ± 468	3237 ± 456	.87
Operative bleeding (mL)	277 ± 44	350 ± 17	.06
Postpartum bleeding (mL)	401 ± 50	461 ± 122	.13

The autophagosome in human uterine myometrium, which was chosen randomly from the biopsy, was investigated by TEM. As shown in Figure 1A, autophagic vacuoles were seen in the myometrium in both groups containing intracytoplasmic organelles. The electron microscopic analysis also showed some vacuolar mitochondria in the in-labor group (d). We quantified the autophagosome between the 2 groups (Figure 1B), and there were significantly more autophagosomes in the in-labor group than in the nonlabor one ($P < .01$).

Microtubule-associated protein 1 light chain 3 is a reliable marker of autophagosome formation in mammalian cells, which is recruited specifically to the autophagosomal membrane. When the autophagy is activated, LC3 is subsequently processed to LC3-I (cytosolic form) and modified to the active LC3-II form (membrane bound form) by posttranslational modification. LC3-II after conjugation and lipidation with phosphatidylethanolamine binds to the outer membrane of autophagosomes. So, LC3II/I ratio is indicative of autophagic activity.[13] LC3B is one of 3 LC3 isoforms (LC3A, LC3B, and LC3C) and can be used as an autophagy marker.[13] P62 is an adaptor molecule implicated in the targeting of cargo for autophagosomes degraded by autophagy,[14] and Beclin-1 is part of an early complex that promotes synthesis and growth of preautophagosomal membranes.[14,19]

Using RT-PCR to evaluate messenger RNA (mRNA) level of these markers, we observed a significantly higher level of LC3B in the in-labor group compared with the nonlabor group (Figure 2, $P < .05$). In contrast to LC3B, P62 mRNA expression was significantly decreased in the in-labor group versus the nonlabor group (Figure 2, $P < .01$). Beclin-1 performs a critical

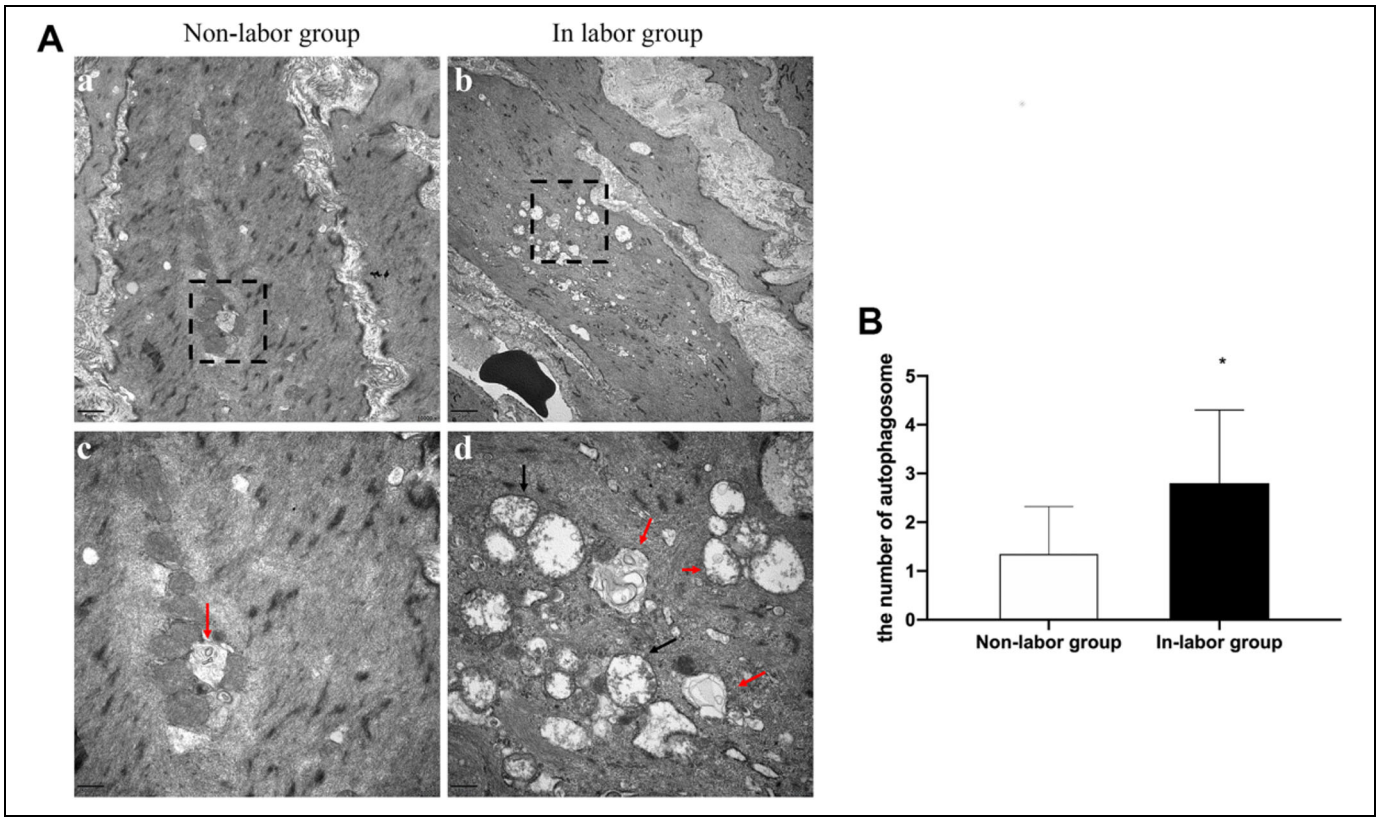

Figure 1. Electron microscopy images of human uterine myometrium. Transmission electron microscopy (TEM) images (A) demonstrated autophagic vacuolose in the nonlabor and in-labor groups. The lower panel (C and D) showed the magnified image of the area indicated by the box in the upper panel (A and B). The quantitative analysis showed there were significantly much more autophagosomes in the in-labor group than in the nonlabor one (B, n = 10; *$P < .05$, 1-way analysis of variance [ANOVA]). Each slice was chosen 5 views randomly under the 500 μm electron microscopy, and calculated the number of autophagosome. The autophagic vacuoles were marked by red arrows. Vacuolar mitochondria were marked by black arrows (D). Scale bar in (A) 1 μm; (B) 2 μm; (C) and (D) 500 nm.

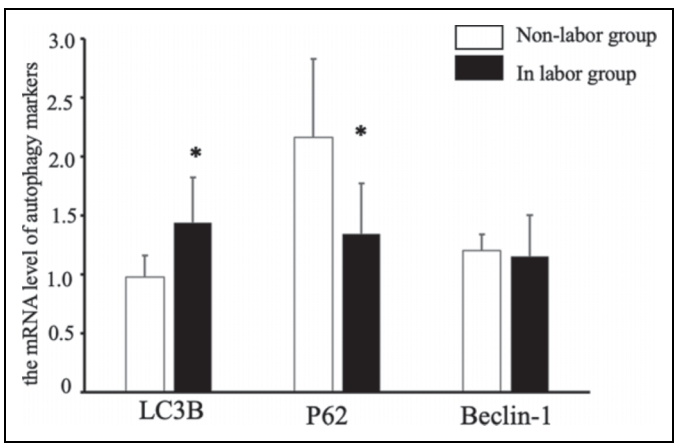

Figure 2. The messenger RNA (mRNA) expression of autophagy markers in human uterine myometrium between groups. It showed mRNA levels of both groups. The in-labor group had significantly greater LC3B mRNA expression but significantly lower P62 mRNA expression compared with the nonlabor group (n = 10, *$P < .05$, 1-way analysis of variance [ANOVA]). But the mRNA expression of Beclin-1 was not significantly different between the 2 groups (n = 10, $P = .247$, 1-way ANOVA).

role not only in autophagosome formation but also in autophagy and apoptosis.[19] The RT-PCR results showed there was no difference between the groups ($P = .247$).

Immunofluorescence and Western blot were used to evaluate the protein level of autophagy markers. The Western blot results showed that LC3B II/I ratio was increased in the in-labor group (Figure 3, $P < .01$), while P62 protein expression level was decreased (Figure 3, $P < .01$). Differences for all parameters were significantly different, except Beclin-1 protein expression ($P = .118$).

Immunofluorescence images (Figures 4A, 5A, and 6A) confirmed LC3B, P62, and Beclin-1 protein expression in human uterine myometrium. Quantitative analysis showed that the fluorescence density of LC3B and Beclin-1 was significantly increased in the in-labor group ($P < .01$, Figure 4B; $P < .01$, Figure 6B), but the density of P62 was significantly decreased ($P < .01$, Figure 5B).

Discussion

Our study demonstrated that normal labor was associated with increased expression of autophagy-associated moieties. To our

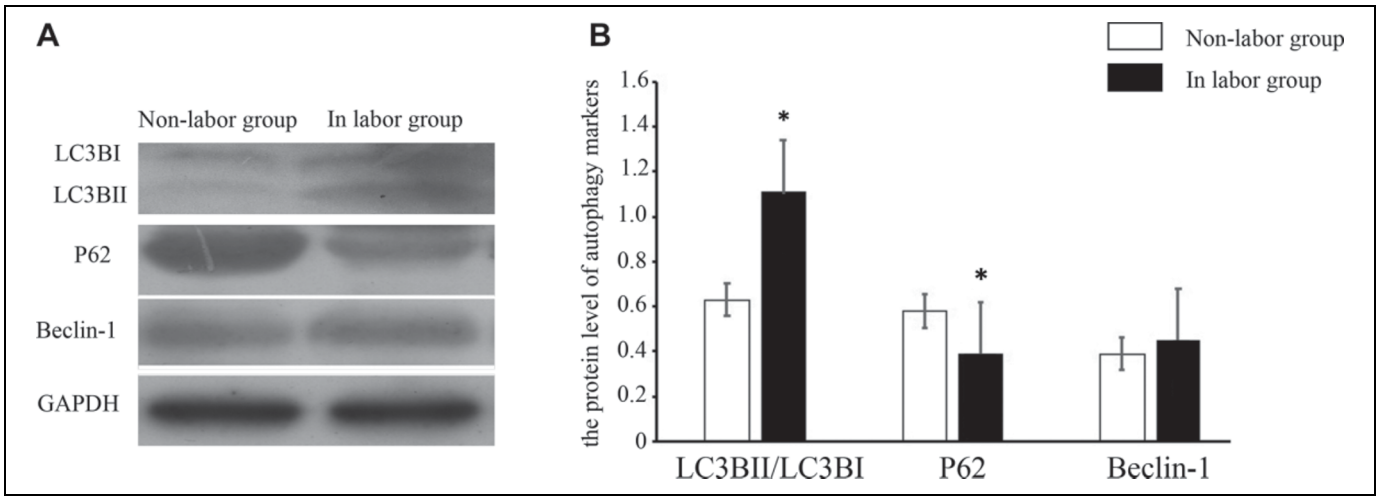

Figure 3. The protein expression of autophagy markers in human uterine myometrium between groups. A and B, The protein level of LC3BII/I, P62, and Beclin-1. The in-labor group had significantly higher protein level of the LC3BII/I ratio and significantly lower P62 protein level compared with the nonlabor group (n = 10, *$P < .05$, 1-way analysis of variance [ANOVA]). The expression of Beclin-1 was not significantly different between the 2 groups (n = 10, $P = .118$, 1-way ANOVA).

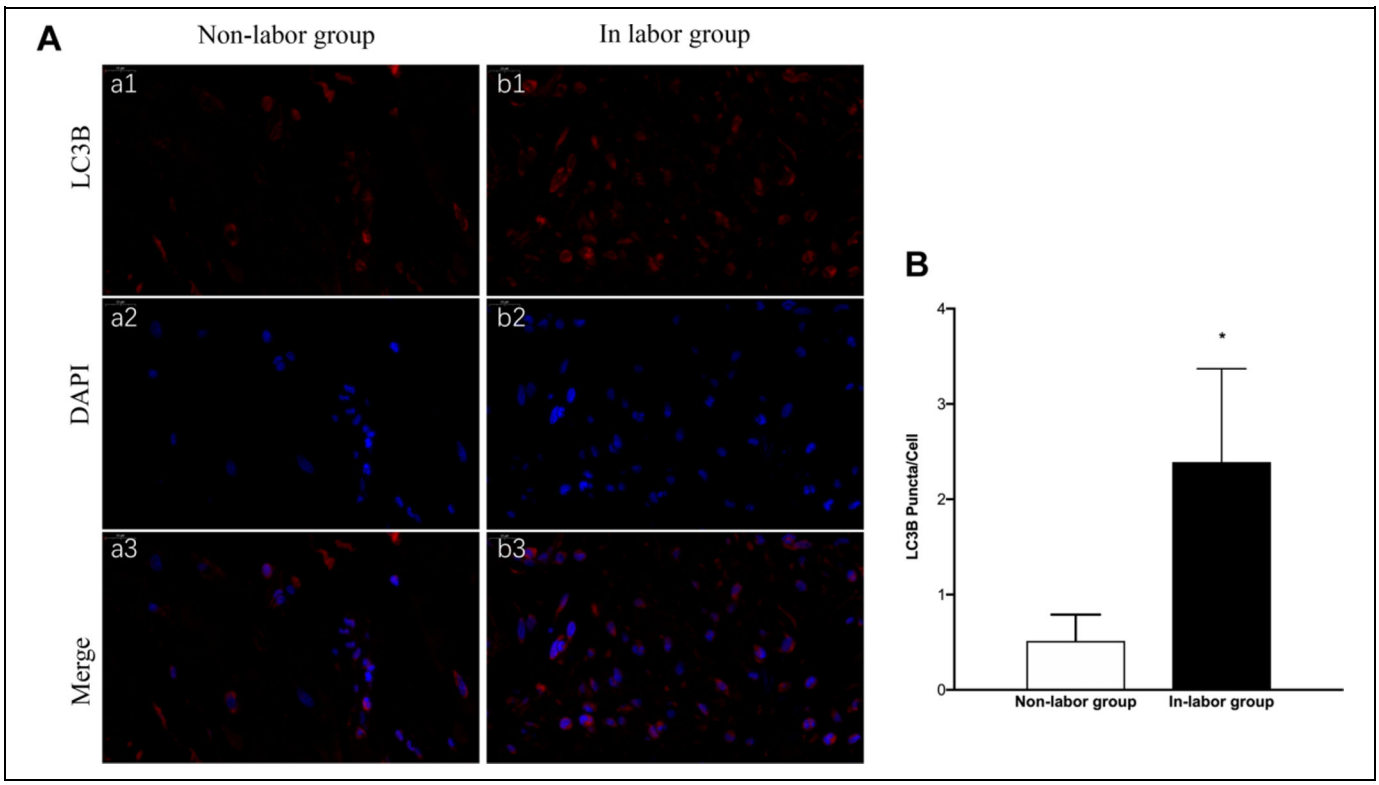

Figure 4. The immunofluorescence of LC3B in human uterine myometrium between groups. Immunofluorescence demonstrated a significantly higher intensity of LC3B (red puncta) in the myometrium (a1, b1) in the in-labor group compared with the nonlabor group (B; n = 10, *$P < .01$, 1-way analysis of variance [ANOVA], scale bar = 20 μm).

knowledge, this is the first study exploring the biology of autophagosomes and autophagy-related proteins in human myometrium during labor. Electron microscopy demonstrated evidence of autophagy activation in human myometrium during labor. We also assessed the mRNA and protein levels of several autophagy markers. Our results suggest that autophagy is activated during labor and perhaps could be one of the mechanisms that maintains uterine contractions during labor.

Many studies have shown that autophagy is intimately involved in all stages of mammalian reproduction from gametogenesis to parturition and associates with many pregnancy-related diseases.[20] For the placenta, it is critical for its

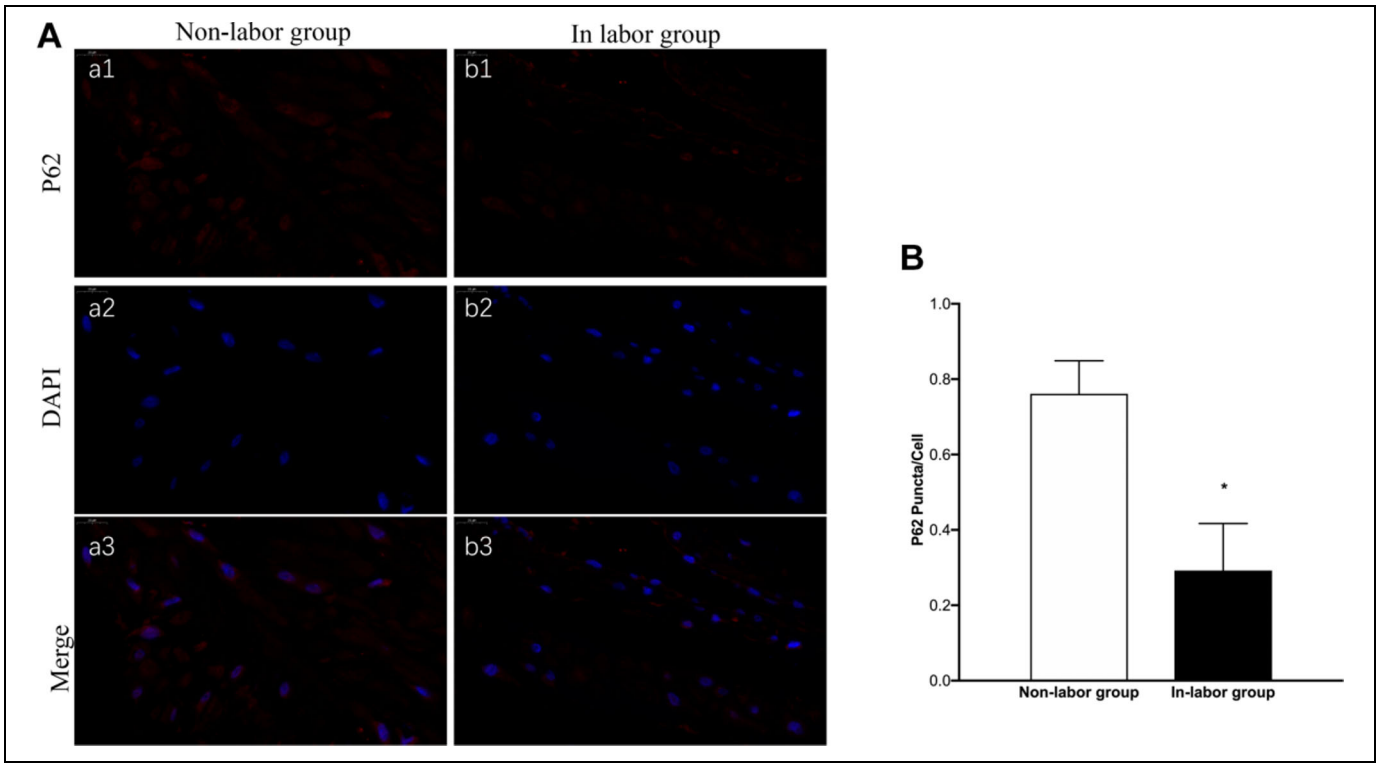

Figure 5. The immunofluorescence of P62 in human uterine myometrium between groups. Immunofluorescence demonstrated P62 located in human myometrium (a1, b1). The intensity of P62 (red puncta) was decreased in the in-labor group compared with the nonlabor group (B; n = 10, *P < .01, 1-way analysis of variance [ANOVA], scale bar = 20 μm).

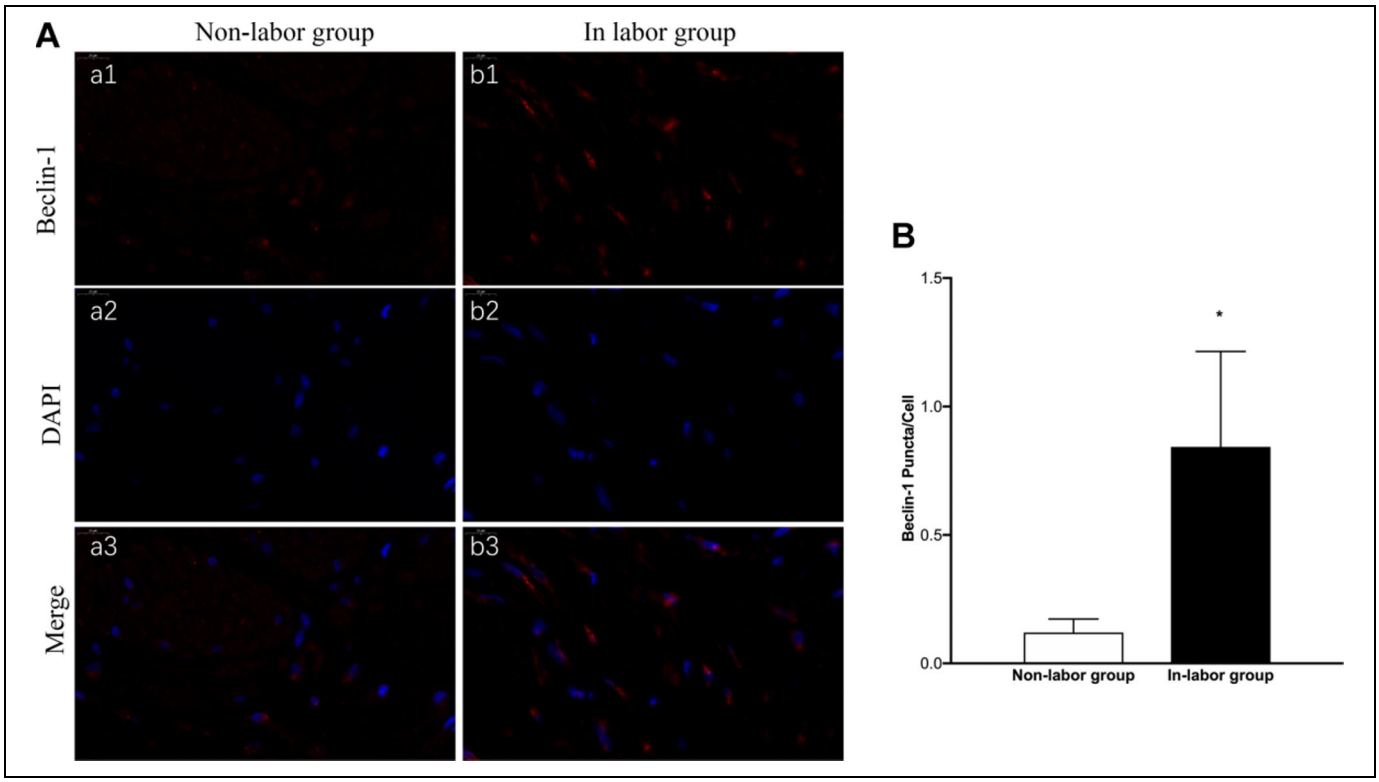

Figure 6. The immunofluorescence of Beclin-1 in human uterine myometrium between groups. Immunofluorescence demonstrated that Beclin-1 localized to the myometrium (a1, b1) and was increased in the in-labor group compared with the nonlabor group (B; n = 10, *P < .01, 1-way analysis of variance [ANOVA], scale bar = 20 μm).

development, protects trophoblasts from apoptosis induced by hypoxia and nutrition deprivation, and maintains a cellular bioenergetic balance between anabolic and catabolic processes.[20-22] However, impaired autophagy in extravillous trophoblast cells contributes to the pathophysiology of preeclampsia.[23] Some studies about the preterm birth revealed that elevated cyclooxygenase 2 and prostaglandin F synthase levels led to inhibit autophagy and senesce decidua, which induced preterm birth.[24] The dysregulation of autophagy before term may be a trigger for premature parturition. But our study showed that the autophagic flux was activated during the parturition in human uterine myometrium as LC3B increasing meanwhile P62 decreasing. Maybe during the labor, the hypoxia metabolic products were specificity recognized by the substrate receptor and degraded by the autophagosome.

During labor, the uterine myometrium undergoes brief episodes of hypoxia and reoxygenation.[12] A previous study showed that in the uterine myometrium, hypoxia-induced force increase is a novel mechanism underlying the strengthening of labor contractions.[12] Another study by Alotaibi also showed that repeated episodes of brief hypoxia could protect and improve uterine contractility after prolonged hypoxia in the uterus in full-term pregnancies.[25] In several tissues, these brief, repeated episodes of hypoxia may improve tolerance against deleterious effects of hypoxia, a phenomenon that has been called "hypoxic preconditioning".[15,26,27] It appears that autophagy is activated during preconditioning and may mediate protection against ischemic insult.[15] Gurusamy et al[27] and Yan et al[28] first showed that autophagy is involved in the protection of cardiac muscle induced by ischemia preconditioning. According to their studies, the expression of the autophagy-related protein LC3 and Beclin-1 promoted the formation of autophagosomes. Autophagy has also been associated with a protective role in liver ischemic preconditioning.[29] During parturition, uterine contractions compress the uterine arteries and reduce myometrial blood flow. This phenomenon may be similar to hypoxic/ischemic preconditioning. Our study demonstrated that autophagy was activated in labor and may help protect the myometrium against damage from long periods of uterine contractions.

At the same time, some studies have suggested that autophagy functions as a protective pathway during ischemia and hypoxia.[15] It is well studied in many organs, such as heart and brain. In 2005, Yan first reported that repetitive myocardial ischemia triggered autophagy via the increased expression of LC3 in pigs.[30] Carloni et al agreed that activation of autophagic pathways is a possible protective mechanism in the early stage of the brain ischemia.[31,32] Activation of autophagy leads to degradation of damaged organelles, maintains energy supply, and promotes cell survival.[15] In skeletal muscle, increased autophagy is an adaptive mechanism during exercise, the main oxidative stress in that tissue, to accelerate the removal of muscle oxidative damage in the organelles or protein, improve metabolism, and maintain glucose homeostasis.[33] In vivo experiments demonstrated that uterine contractions produce a decrease in intracellular pH and adenosine triphosphate, and lactate accumulation in myometrium.[34] These metabolic events may inhibit uterine contractions.[34,35] Our study suggested that during normal labor autophagy increases, which degrades metabolic products to preserve energy and metabolic homeostasis, in an effort to maintain contraction strength and myometrial function.

Hypoxia is an autophagy-inducing stimulus. Signaling to regulate autophagy during ischemia and preconditioning is very complicated, involving AMPK/TSC/mTOR, Beclin-1/BINP3/SPK2, protein kinase C-dependent autophagy, and FoxO/NF-κB.[14] Our study has demonstrated that activation of autophagy in human myometrium occurred during normal labor and that mRNA and protein levels were increased for LC3B and Beclin-1 but decreased for P62. But how autophagy plays a role in human uterine myometrium suffering repeated hypoxia during labor is still not clearly elucidated. Studies involving pathological conditions and clinical intervention may help clarify how autophagy helps maintain uterine muscle contraction in labor.

In conclusion, autophagy is activated in human uterine smooth muscles during the labor in which the utero contracts progressively stronger while suffering brief hypoxia repeatedly. Our study suggested that autophagy might play an important role in maintaining uterine contraction function. Future work could lead to clarify the mechanism through pathological conditions and clinical intervention.

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Declaration of Conflicting Interests

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References

1. Arrowsmith S, Kendrick A, Hanley JA, Noble K, Wray S. Myometrial physiology—time to translate? *Exp Physiol*. 2014;99(3):495-502.
2. Smith R, Imtiaz M, Banney D, Paul JW, Young RC. Why the heart is like an orchestra and the uterus is like a soccer crowd. *Am J Obstet Gynecol*. 2015;213(2):181-185.
3. Chaemsaithong P, Madan I, Romero R, et al. Characterization of the myometrial transcriptome in women with an arrest of dilatation during labor. *J Perinat Med*. 2013;41(6):665-681.
4. Cohen WR, Friedman EA. The assessment of labor: a brief history. *J Perinat Med*. 2018;46(1):1-8.

5. Mylonas I, Friese K. Indications for and risks of elective cesarean section. *Dtsch Arztebl Int*. 2015;112(29-30):489-495.
6. Brar HS, Platt LD, DeVore GR, Horenstein J, Medearis AL. Qualitative assessment of maternal uterine and fetal umbilical artery blood flow and resistance in laboring patients by Doppler velocimetry. *Am J Obstet Gynecol*. 1988;158(4):952-956.
7. Fleischer A, Anyaegbunam AA, Schulman H, Farmakides G, Randolph G. Uterine and umbilical artery velocimetry during normal labor. *Am J Obstet Gynecol*. 1987;157(1):40-43.
8. Baron J, Shwarzman P, Sheiner E, et al. Blood flow doppler velocimetry measured during active labor. *Arch Gynecol Obstet*. 2015;291(4):837-840.
9. Larcombe-McDouall J, Buttell N, Harrison N, Wray S. In vivo pH and metabolite changes during a single contraction in rat uterine smooth muscle. *J Physiol*. 1999;518(pt 3):783-790.
10. Delbridge LMD, Mellor KM, Taylor DJ, Gottlieb RA. Myocardial stress and autophagy: mechanisms and potential therapies. *Nat Rev Cardiol*. 2017;14(7):412-425.
11. Wolf MS, Bayır H, Kochanek PM, Clark RSB. The role of autophagy in acute brain injury: a state of flux? *Neurobiol Dis*. 2019; 122(2):9-15.
12. Alotaibi M, Arrowsmith S, Wray S. Hypoxia-induced force increase (HIFI) is a novel mechanism underlying the strengthening of labor contractions, produced by hypoxic stresses. *Proc Natl Acad Sci U S A*. 2015;112(31):9763-9768.
13. Klionsky DJ, Abdelmohsen K, Abe A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy*. 2016;12(1):1-222.
14. Antonioli M, Di Rienzo M, Piacentini M, Fimia GM. Emerging mechanisms in initiating and terminating autophagy. *Trends Biochem Sci*. 2017;42(1):28-41.
15. Yan WJ, Dong HL, Xiong LZ. The protective roles of autophagy in ischemic preconditioning. *Acta Pharmacol Sin*. 2013;34(5): 636-643.
16. Sheng R, Qin ZH. The divergent roles of autophagy in ischemia and preconditioning. *Acta Pharmacol Sin*. 2015;36(4):411-420.
17. Oh SY, Choi SJ, Kim KH, Cho EY, Kim JH, Roh CR. Autophagy-related proteins, LC3 and Beclin-1, in placentas from pregnancies complicated by preeclampsia. *Reprod Sci*. 2008;15(9):912-920.
18. Li X, Han X, Bao J, et al. Nicotine increases eclampsia-like seizure threshold and attenuates microglial activity in rat hippocampus through the α7 nicotinic acetylcholine receptor. *Brain Res*. 2016;1642:487-496.
19. Luo S, Rubinsztein DC. Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-xL. *Cell Death Differ*. 2010;17(2):268-277.
20. Kanninen TT, de1Andrade Ramos BR, Witkin SS. The role of autophagy in reproduction from gametogenesis to parturition. *Eur J Obstet Gynecol Reprod Biol*. 2013;171(1):3-8.
21. Saito S, Nakashima A. Review: the role of autophagy in extravillous trophoblast function under hypoxia. *Placenta*. 2013; 34(suppl):S79-S84.
22. Oh SY, Choi SJ, Kim KH, et al. Autophagy-related proteins, LC3 and Beclin-1, in placentas from pregnancies complicated by preeclampsia. *Reprod Sci*. 2008;15(9):912-920.
23. Nakashima A, Yamanaka-Tatematsu M, Fujita N, et al. Impaired autophagy by soluble endoglin, under physiological hypoxia in early pregnant period, is involved in poor placentation in preeclampsia. *Autophagy*. 2013;9(3):303-316.
24. Hirota Y, Daikoku T, Tranguch S, Xie H, Bradshaw HB, Dey SK. Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. *J Clin Invest*. 2010; 120(3):803-815.
25. Alotaibi M. Brief hypoxic cycles improve uterine contractile function after prolonged hypoxia in term-pregnant but not in non-pregnant rats in vitro. *Theriogenology*. 2018;113:73-77.
26. Zhou P, Tan YZ, Wang HJ, Wang GD. Hypoxic preconditioning-induced autophagy enhances survival of engrafted endothelial progenitor cells in ischaemic limb. *J Cell Mol Med*. 2017; 21(10):2452-2464.
27. Gurusamy N, Lekli I, Gorbunov NV, Gherghiceanu M, Popescu LM, Das DK. Cardioprotection by adaptation to ischaemia augments autophagy in association with BAG-1 protein. *J Cell Mol Med*. 2009;13(2):373-387.
28. Yan L, Sadoshima J, Vatner DE, Vatner SF. Autophagy in ischemic preconditioning and hibernating myocardium. *Autophagy*. 2014;5(5):709-712.
29. Domart MC, Esposti DD, Sebagh M, et al. Concurrent induction of necrosis, apoptosis, and autophagy in ischemic preconditioned human livers formerly treated by chemotherapy. *J Hepatol*. 2009; 51(5):881-889.
30. Yan L, Vatner DE, Kim SJ, et al. Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci U S A*. 2005; 102(39):13807-13812.
31. Carloni S, Buonocore G, Balduini W. Protective role of autophagy in neonatal hypoxia-ischemia induced brain injury. *Neurobiol Dis*. 2008;32(3):329-339.
32. Carloni S, Buonocore G, Longini M, Proietti F, Balduini W. Inhibition of rapamycin-induced autophagy causes necrotic cell death associated with Bax/Bad mitochondrial translocation. *Neuroscience*. 2012;203:160-169.
33. Neel BA, Lin Y, Pessin JE. Skeletal muscle autophagy: a new metabolic regulator. *Trends Endocrinol Metab*. 2013;24(12): 635-643.
34. Hanley JA, Weeks A, Wray S. Physiological increases in lactate inhibit intracellular calcium transients, acidify myocytes and decrease force in term pregnant rat myometrium. *J Physiol*. 2015;593(20):4603-4614.
35. Quenby S, Pierce SJ, Brigham S, Wray S. Dysfunctional labor and myometrial lactic acidosis. *Obstet Gynecol*. 2004;103(4):718-723.