

## Long Non-Coding RNAs, Ubiquitin Proteasome System, Collagen Degradation and Preterm Premature Rupture of Membrane

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### Abstract

Preterm Premature Rupture of the Fetal Membranes (PPROM) is a reproductive system disorder and a major cause of prematurity. Several major etiologic factors have been linked to PPROM, one of which is the weakness of the amniochorion Extra Cellular Matrix (ECM) caused by collagen degradation. With increasingly deeper research studies on the human genome, rapidly growing evidence has suggested that abnormally expressed non-coding RNAs (ncRNAs) are involved with multiple diseases. Among various ncRNAs, the long non-coding RNAs (lncRNAs) have attracted more attention and were found to correlate with various inflammation-related conditions or diseases. Recent studies demonstrated that lncRNAs might be involved in regulation of the ubiquitin proteasome system (UPS) in PPROM. The UPS is an ATP-dependent enzyme process that targets substrate proteins, tagged with an isopeptide chain composed of covalently linked molecules of ubiquitin, for degradation by the 26S proteasome, and deeply involved in the regulation of most basic cellular processes. Here, we reviewed the UPS system, the collagen in extracellular matrix (ECM), the PPROM as well as lncRNAs. We hypothesize that a novel pathogenic pathway of "infection/inflammation  $\Rightarrow$  lncRNA  $\Rightarrow$  UPS  $\Rightarrow$  collagen  $\Rightarrow$  membrane rupture" for exploring the molecular pathogenesis of PPROM.

**Keywords:** Collagen degradation; RNAs; PPROM; Extra cellular matrix

### Introduction

Preterm Premature Rupture of the fetal Membranes (PPROM) is a reproductive system disorder, which is the major cause of prematurity [1]. Several major etiologic factors have been linked to PPROM, one of which is the weakness of the amniochorion Extra Cellular Matrix (ECM) caused by collagen degradation. The omnipresent ubiquitin proteasome system (UPS) is an ATP-dependent enzymatic system that targets substrate proteins, which were tagged with an isopeptide chain composed of covalently linked molecules of ubiquitin, for degradation by the 26S proteasome [2]. This system is deeply involved in the regulation of most basic cellular processes, and deregulation of UPS can result in certain kinds of human diseases. Moreover, UPS has been demonstrated to exist extracellularly and regulate the degradation of extracellular proteins [3]. However, there has been no previous effort made to explore the possible pathogenic role of UPS in PPROM. Here, we reviewed the current studies on UPS and PPROM, especially the possible mechanism of UPS regulating collagens of the ECM in PPROM.

### Ubiquitin proteasome system

**Components of UPS:** The UPS is the principal pathway for clearing short-lived, damaged, and misfolded proteins in the nucleus and cytoplasm [4]. This system contained two separate and consecutive steps: ubiquitylation, which involved the process ubiquitins were covalently attached to substrate protein, and proteasomal degradation, in which the ubiquitin-labeled proteins were degraded by proteasome [2]. Ubiquitin is a highly conserved protein with 76 amino acids, which was discovered as a macromolecular tag [5]. In mammals, ubiquitin is encoded by four different genes (*UBB*, *UBC*, *UBA52*, and *UBA80*) that are tightly regulated by various transcriptional and posttranslational mechanisms to maintain adequate free ubiquitin concentrations in cells [6]. In the ubiquitylation process, ubiquitins are attached to protein, which needs three steps: the ATP-dependent activation of ubiquitin by a ubiquitin-activating enzyme (E1), transfer of activated ubiquitin to a ubiquitin-conjugating enzyme (E2) and then formation of an isopeptide bond between ubiquitin and the substrate protein catalysed

by a ubiquitin-ligase (E3) [7]. The process is repeated several times with the aim to build up a poly-ubiquitin chain by interubiquitin linkages. E1 ubiquitin-activating enzymes can form a high-energy thioester bond between the C-terminus of ubiquitin and cysteine residues in E1. Two E1 enzymes (UBA1 and UBA6) have been found to initiate ubiquitin conjugation in the system [8]. E2 enzymes are responsible for transferring the activated ubiquitin to the E3-substrate complex. In the human genome, E2 enzymes were encoded by about 40 genes, which are the main determinants of poly ubiquitin chain linkage specificity and the chain length on target substrates [9]. There are 600 E3 ubiquitin-ligases encoded in the human genome [10]. The abundance and specificity of the currently identified E3 ligases suggest that E3 enzymes determine the substrate selectivity of the UPS through specific mechanisms recognizing their target substrates [11,12]. Ubiquitylation can also be reversed by de-ubiquitylating enzymes (DUBs) that remove ubiquitin from proteins and disassemble multiubiquitin chains. The activity of DUBs provides an additional level of regulatory control and maintains a sufficient pool of free ubiquitin molecules in the cell by removing the ubiquitin tag from degraded proteins [13].

In the proteasomal degradation process, proteins modified by polyubiquitin chains are bound and cleaved into short peptides by the 26S proteasome. The 26S proteasome is a 2-MDa ATP-dependent proteolytic complex that degrades ubiquitin conjugates [14,15]. It contains 31 principal subunits arranged into two subcomplexes, the core catalytic 20S proteasome (CP) and the 19S regulatory particle (RP)

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[16,17]. CP is a broad spectrum of ATP- and ubiquitin-independent protease formed by 28 subunits arranged in four heptameric stacked rings, with peptidase activity localized to the inner  $\beta$  rings [18]. RP is important for regulating the activity of the 26S proteasome complex [19]. It associates with one or both ends of the CP and confers both ATP dependence and specificity for Lys48-linked polyubiquitin chains to the particle [14,15]. The RP is composed of 17 core subunits that can be further divided into the Lid and Base subcomplexes [20]. The Lid contains the remaining non-ATPase subunits (RPN3, 5-9, and 11-12) resembling the COP9 signalosome [21]. The Base contains six AAA-type ATPases (RPT1-6) and three non-ATPase subunits (RPN1, 2 and 10), functioning as a molecular motor unfolding and translocating the protein substrate [22]. When a protein is modified by a polyubiquitin chain of at least four Lys48-linked ubiquitins, it can bind either directly to intrinsic ubiquitin receptors in the 19S complex or to adaptor proteins that link it to the 19S complex [23]. Following binding to the proteasome, the protein is unfolded by ATPase and removed from the polyubiquitin chain by proteasome-associated DUBs. Then the unfolded protein is translocated into the central proteolytic chamber of the 20S subunit, where it is cleaved into short peptides [23]. Through the degradation mechanism above, UPS could be involved in various cellular processes, such as regulation of gene expression [24], protein cell cycle control [25], regulation of signal transduction [26,27], and mitochondrial intermembrane function [28]. Furthermore, dysfunction of UPS have been associated with many human complex diseases, such as different cancers [29,30], cardiovascular disease [31], neurodegenerative disease [32-34], and kidney disease [35]. The more recent studies even found that UPS were implicated in aging [36,37].

**Extracellular UPS:** It is known that the major part of UPS is located within the cytoplasmic and nuclear compartments. Therefore, the UPS is generally considered as the major pathway for intracellular protein degradation. However, increasing evidence has supported that elements of UPS could exist extracellularly, which may suggest the novel extracellular function of UPS (in organisms) [3]. The core catalytic 20S proteasome has been found to be attached to the cell plasma membrane [38], and certain investigations suggest that they may be released into the extracellular medium, such as the alveolar lining fluid [39], epididymal fluid [40], cerebrospinal fluid [41], and possibly during the acrosome reaction [42]. Proteasomes have also been detected in the alveolar lining fluid as well as human blood plasma and have been designated as circulating proteasomes [39,43]. The concentration of circulating proteasomes has been found to be elevated in patients suffering from autoimmune diseases, malignant myeloproliferative syndromes, multiple myeloma, acute and chronic lymphatic leukaemia, solid tumour, sepsis or trauma [43-46]. Moreover, it was demonstrated that enzymatic activity of the 20S subunit of proteasomes was positive in cerebrospinal fluid of healthy individuals by the fluorescent substrate cleavage [41]. Except for proteasomes, ubiquitin has also been discovered as a normal component in human blood, seminal plasma and even ovarian follicular fluid [3]. Increased systemic levels of extracellular ubiquitin have also been observed in several very different diseases like alcohol-induced liver cirrhosis and brain atrophy, type 2 diabetes, chronic hemodialysis, hairy cell leukaemia, sepsis and severe trauma [47-52]. The available evidence has indicated that these extracellular components could compose the extracellular UPS that could be involved in the regulation of extracellular proteins. For example, several researchers have found that the extracellular UPS could control fertilization through ubiquitination and degradation of the vitelline coat during human and animal fertilization [53,54].

## PPROM

### Histological structure of fetal membrane

Human fetal membrane, lining the intrauterine cavity, consists of amnion and chorion connected by an ECM [55]. This membranous layer provides the sac in which fetal growth takes place, and grows as gestation progresses to accommodate the increasing volume of the fetus and amniotic fluid [56]. The amniotic epithelium is the innermost layer, which directly contacts the amniotic fluid. The amnion consists of both epithelial and mesenchymal components [56]. The columnar or cuboidal epithelial-cell layer lines the amniotic cavity. The chorion is formed from the implanted blastocyst at the pole towards the endometrial cavity that is covered by chorion frondosum and decidua capsularis. The blood supply to this area becomes restricted and villi degenerate, forming an avascular chorion. Amnion fuses with the mesoderm of the chorion to form the chorioamnion. The remnants of the capsular decidua adhering to the chorion become opposed to the maternal parietal decidua [57].

The ECM is composed of a large collection of biochemically distinct components including proteins, glycoproteins, proteoglycans, and polysaccharides with different physical and biochemical properties [58-60]. Structurally, these components make up both basement membrane, which is produced jointly by epithelial, endothelial, and stromal cells to separate epithelium or endothelium from stroma, and interstitial matrix, which is primarily made by stromal cells. The basement membrane is a specialized ECM, which is more compact and less porous than interstitial matrix. It has a distinctive composition containing type IV collagen, laminins, fibronectin, and linker proteins such as nidogen and entactin, which connect collagens with other protein components. In contrast, interstitial matrix is rich in fibrillar collagens, proteoglycans, and various glycoproteins such as tenascin C and fibronectin and is thus highly charged, hydrated, and contributes greatly to the tensile strength of tissues [60]. In fetal membrane, ECM is composed of fibrous proteins embedded in a polysaccharide gel, which forms the architectural framework of the amniochorion [61]. Collagens are important structural elements of ECM which determine the tensile strength of the membrane. They form the major structural framework of the fetal membrane ECM [57,62,63]. The types of collagen in fetal membrane include I, III, IV, V, VI and VII. The major tensile strength is provided by interstitial collagens types I and III together with small amounts of types V, VI and VII. The type IV collagen located in the basement membrane connects the amnion and chorion to the ECM, which provides the scaffold for the assembly of other non-collagen structural proteins and plays a major role in the development and maintenance of the ECM [63]. Types V and VII are minor fibrillar collagen which provide an additional anchoring function for the basement membrane along with type IV collagen. Types VI and VII are present in smaller quantities in the fetal membrane ECM; however, along with types I and III, they form an anchoring fibrillar structure. In addition, except for different types of collagen, other components of the ECM also include laminin, elastin, proteoglycan, microfibrils, fibronectin, decorin, plasminogen and integrins [57,63,64]. The ECM collagens undergo constant turnover and remodeling throughout pregnancy to accommodate the increasing volume and tension as gestation progresses [65]. This remodeling process results in a decreased collagen content of the amnion in the last eight weeks of pregnancy [65].

### PPROM and collagen degradation

PPROM, defined as a rupture of the membranes occurring before 37 weeks of gestation, is one of the major causes of prematurity,

accounting for 30-40% of all preterm births [1]. Several major etiologic factors have been linked to PPRM, such as maternal reproductive tract infection (bacterial vaginosis, trichomoniasis, gonorrhea, chlamydia, and subclinical chorioamnionitis), behavioral factors (smoking, substance abuse, nutritional status, and coitus), obstetric complications (multiple gestation, polyhydramnios, insufficient cervix, cervical operations, gestational bleeding, and antenatal trauma), and possibly environmental changes (barometric pressure) [56]. These factors can result in weakness of the amniochorion ECM by collagen degradation, which is one of the key events predisposing to membrane rupture [65]. Among these, infection is one of the most common etiological factors causing spontaneous preterm birth and PPRM, and usually involves cytokine/chemokine pathways and degradation of ECM [66-71], causing an increased matrix MMPs and decreased tissue inhibitors of MMP (TIMP) [72]. The changes of MMPs and TIMP can result in collagenolysis and reduction of the collagen content of fetal membrane, as seen in spontaneous preterm birth and PPRM [72]. Indeed, a decrease in total collagen content and an increase in collagen solubility, and an increase in collagenolytic activity that results in the remodeling of the extracellular matrix have been the characteristics of the cervical softening and decidual and fetal membrane activation [73].

Collagen degradation can be activated by exogenous and endogenous factors. The exogenous factors include the effects of bacterial metabolism and maternal or fetal host inflammatory response. Although bacterial collagenases have been found in the amniotic fluid during PPRM, they are neither specific nor produced in sufficient quantities to effectively degrade human collagens. Bacterial infection is more like an initiator while the host inflammatory response is the true causative agent in PPRM. The host inflammatory response initiated by bacteria or bacterial components (LPS) activates ECM collagen-specific matrix metalloproteinases (MMPs) that lead to ECM degradation through degrading collagens in ECM, predisposing the fetal membrane to rupture [56]. It was reported that MMP1 and MMP8 are collagenases that act to degrade collagen types I, II, which are up-regulated in the amnion and chorion in PPRM [74]. The MMPs may be stimulated by intrauterine inflammatory infection, and bacteria or bacterial product may directly stimulate MMP productions [72,75]. Moreover, the major proinflammatory cytokines (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ) produced in host inflammatory response can promote PPRM by inducing apoptosis [56]. The endogenous factors include a local variation in membrane thickness and a reduction in collagen content, which may be influenced by genetic predispositions. Since PPRM is a complex disease and involves multiple pathophysiologic pathways, gene-gene interactions and gene-environmental interactions may play important roles in PPRM. Single-nucleotide polymorphisms (SNPs) of several candidate genes (MMP-8, MMP 9, TNF- $\alpha$ , Fas, and HSP70) involved in the already identified PPRM pathways are assumed to associate with PPRM [76-80]. In addition, several studies have found that variants in genes encoding collagens are involved in human disease. For example, polymorphism of 1997G  $\rightarrow$  T in the promoter of the collagen type I gene was associated with bone mineral density for the lumbar spine in postmenopausal Spanish women [81]. A variant within COL5A1 encoding a subunit of type V collagen was correlated with injury and performance phenotypes [82]. The rs2621215 SNP in intron 46 of the COL1A2 gene was found to be marginally associated with an increased risk of developing intracranial aneurysms in the Korean population [83]. Sequence variants within the 3'-UTR of the COL5A1 gene could alter mRNA stability which was implicated in musculoskeletal soft tissue injuries [84]. Furthermore, in the molecular basis study of musculoskeletal soft tissue injuries and other exercise-related phenotypes, a functional miRNA site for Hsa-miR-608 within

the COL5A1 3'-UTR was identified and additional elements regulating COL5A1 mRNA stability were also identified using deletion constructs [84]. Considering that collagen degradation is one of the key events predisposing to membrane rupture in PPRM, genetic heterogeneity of collagen genes may affect the occurrence of PPRM in different individuals though rarely research has reported genetic variants in genes encoding different collagens was involved in PPRM up to now. These exogenous and endogenous factors affecting collagen degradation may suggest environmental and genetic factors were interacting in PPRM. Although the molecular mechanism of this interaction is not yet clear, it is hypothesized that the epigenetic regulatory mechanism may play important roles in PPRM and therefore deserves further investigation.

## Epigenetic regulation of noncoding RNA with UPS as well as with PPRM

### Noncoding

**RNAs:** Current high-throughput transcriptomic research has found that eukaryotic genomes transcribe up to 90% of the genomic DNA to RNAs [85]. Among these genomic transcripts, only 1-2% are translated to proteins while the vast majority are identified as non-coding RNAs (ncRNAs) that are defined by lack of protein-coding sequences [85,86]. ncRNAs play important roles in a variety of biological processes [87-89], and can be divided into two major groups according to the length, the short noncoding RNAs, which include microRNAs (miRNA), PIWI-interacting RNA (piRNA), small nucleolar RNAs (snoRNAs) as well as other non-coding transcripts of less than 200 nucleotides (nt), and the more recently described long noncoding RNAs (lncRNA) that are longer than 200nt [90,91].

MiRNAs are the most widely studied class of short noncoding RNAs, which mediate post-transcriptional gene silencing by controlling the translation of mRNA into protein [92,93]. Research has found that miRNAs can be involved in regulation of many biological processes, such as proliferation, differentiation, apoptosis and development [94]. The disruption expression of miRNAs has been found in many human diseases including different cancers, neurological disorders, cardiovascular disorders and others [94]. For example, miR-15 and miR-16 were dysregulated in most B cell chronic lymphocytic leukemia [95]; miR-206 deficiency accelerates amyotrophic lateral sclerosis [96]; miR-1, which is involved in heart development, has been linked with arrhythmias through down-regulating expression of the ion channel genes [97,98]. Except miRNAs, the disruption of other classes of short noncoding RNAs, such as snoRNAs and piRNAs, can also lead to human diseases [94]. For example, the germline homozygous 2 bp (TT) deletion of the snoRNA U50 is associated with prostate cancer development [99], and the overexpression of piRNAs, PIWIL1 and PIWIL2, is involved in kinds of somatic tumours [100-102].

lncRNAs may function as regulators of protein-coding gene expression and exert a variety of intrinsic functions in eukaryocytes [103]. In genomic contexts, lncRNAs can be transcribed from enhancers, promoters, introns of genes, pseudogenes and antisense to genes [104]. They can influence almost every step in the life cycle of genes, and carry out their biological roles through several different mechanisms, including regulating chromatin states and nuclear compartments [105-107], affecting the process of transcription [108-110], and mediating mRNA stability, splicing and translation in post-transcriptional level [111-113]. The disruption of lncRNAs is also found to associate with different human diseases as short noncoding RNAs [114]. For example, ANRIL is the antisense lncRNA of the *INK4* locus, and its altered activity could result in dysregulated silencing of the *INK4* locus,

which contributed to the initiation of several cancers [115-119]. The lncRNA MALAT-1 was associated with early-stage non-small-cell lung cancer [120], which depended on its ability in regulating the alternative splicing through interaction with nuclear phosphoproteins [121,122]. In addition, the antisense lncRNA BACE1-AS, the opposite strand to BACE1, could increase BACE1 mRNA stability and protein abundance on a post-transcriptional level, which was identified as up-regulation in Alzheimer's disease [123]. Moreover, based on screening and expression analyses, multiple lines of evidence increasingly support the linkage of dysfunctions of lncRNAs to other human diseases, such as neurodegenerative and psychiatric diseases [124], cardiovascular disease [125], reproductive disease [126], immune dysfunction and auto-immunity [127]. Recently, more and more studies have identified lncRNAs as novel biomarkers and potential therapeutic targets for human diseases [128]. For example, lncRNA H19 was reported as a novel therapeutic target for pancreatic cancer [129]. Another lncRNA PCAT18 was identified as a novel biomarker and potential therapeutic target for metastatic prostate cancer [130]. In the study of neuropsychiatric disorders, one lncRNA named UBE3A-ATS was identified as a potential therapy target for Angelman syndrome [131].

### Epigenetic regulation of noncoding RNAs with UPS

ncRNAs are not only best known for modulating transcription, but also post-transcriptional influence on mRNA splicing, stability and translation. Recent studies in neurodevelopmental disorders suggest that miRNA can regulate UPS. For example, studies in spinocerebellar ataxia type 1 found that the primary target genes of miRNAs involved in this disease were members of the ubiquitin proteasome system [132]. Another study in neurodevelopmental disorders presented that miR-137 could target the Mind bomb one protein (Mib1), a ubiquitin ligase known to be important for neurodevelopment, through the conserved target site located in the 3' untranslated region of Mib1 mRNA, which has a significant role in regulating neuronal maturation [133]. In addition, in human end-stage dilated cardiomyopathy, miR-199/214 was found to play a significant role in regulatory activity of the UPS by regulating the ubiquitin E2 ligases Ube2i and Ube2g1 [134]. Except for short ncRNAs, evidence for lncRNA regulating UPS was also found. lncRNA HOTAIR was found to act as an inducer of ubiquitin-mediated proteolysis through associating with E3 ubiquitin ligases bearing RNA-binding domains and their ubiquitination substrates [135]. Another nuclear-enriched lncRNA antisense to ubiquitin carboxy-terminal hydrolase L1 (Uchl1) that was one kind of de-ubiquitylating enzymes was identified to increase UCHL1 protein synthesis at a post-transcriptional level in mouse [136]. These findings reveal an undescribed post-transcriptional regulatory pathway of ncRNA to control UPS though more researches are needed to uncover the detailed mechanism.

### Epigenetic regulation of noncoding RNAs in PPRM

Recent studies suggested that ncRNAs were possibly associated with preterm birth (PTB) and PPRM in pregnant women. Two previous microarray studies have implied that multiple miRNAs possibly participated in epigenetic regulation of PTB and PPRM. One microarray study found that the relative expression of 20 miRNAs was differentially expressed in placentas from patients with preeclampsia and preterm birth as compared to normal term, which were involved in miR-15b, miR-181a, miR-200c, miR-210, miR-296-3p, miR-377, miR-483-5p, and miR-493 [137]. Another study using Affymetrix GeneChip miRNA array also identified 99 miRNAs with differential expression in cervical cells between PTB and term birth [138]. Recently, our group firstly reported that lncRNAs were correlated with PPRM

and PTB [139]. Thousands of lncRNAs were differentially expressed in the human placentas of PPRM, PTB, and premature rupture of membrane (PRM) compared with full-term birth (FTB), which illustrated that lncRNAs could be participating in the physiological and pathogenic processes of human pregnancies [139]. Moreover, in our study, 22 lncRNA pathways were characterized as up-regulated and 7 were down-regulated in PPRM vs. PTB, 18 were up-regulated and 15 were down-regulated in PPRM vs. PRM, and 33 were up-regulated and 7 were down-regulated in PPRM vs. FTB. Functional analysis of altered lncRNAs showed infection and inflammatory response to be one major pathogenic mechanism involved in the development of PPRM [139]. Another investigation about lncRNAs have identified co-differential expression and correlation at two genomic loci that contain coding-lncRNA gene pairs: SOCS2-AK054607 and LMCD1-NR024065 in human myometrium in women with spontaneous labor at term [140]. However, the two pairs of mRNA-lncRNA were not found differential expression in our data from the human placentas, which might be explained by the tissue-specific expression of mRNAs and lncRNAs. Although detailed functional mechanism and pathogenesis of how individual miRNAs or lncRNAs play their role(s) in PPRM and PTB are still unknown, these above findings opened a new avenue for exploring epigenetic regulation in PPRM and PTB.

### The Possible Connection between UPS and PPRM

In our previous study, besides lncRNAs, mRNA differential expression was also investigated in human placentas of PPRM, PTB, PRM and FTB [111]. When the combination of PPRM and PRM was compared to that of PTB and FTB, the focus was membrane rupture since both PPRM and PRM share the common feature of premature membrane rupture while PTB and FTB are without membrane rupture. Among the differentially expressed mRNAs we identified, nine UPS-related genes were up-regulated and another ten UPS-related genes were down-regulated when compared to both PPRM and PRM vs. PTB and FTB [111], which suggested that UPS was probably involved in the regulation of membrane rupture in PPRM. Moreover, two collagen-related genes were down-regulated accompanied the changes of UPS-related genes [111]. The weakness of the amniochorion ECM by collagen degradation is one of the key events predisposing to membrane rupture [51]. The UPS is likely to regulate PPRM through control of the collagen content in the amniochorion ECM. Based on the current knowledge reviewed above, UPS may theoretically regulate PPRM through two pathways. First, intracellular UPS may control the production of collagen proteins, which results in concentration changes of collagen in the ECM thus predisposing to membrane rupture. Second, extracellular UPS may directly degrade the collagen of the ECM through the proteasome as MMPs do; the function of extracellular UPS needs to be further confirmed.

Among the multiple epidemiological and clinical findings, maternal reproductive tract infection was considered to be the important promoter of PPRM [56]. The inflammation initiated by infection play primary or secondary roles in the pathogenesis of PPRM. On the one hand, lncRNAs were involved in the regulation of inflammation and immune reaction because the lncRNAs belonging to the relevant pathways were found differentially expressed in PPRM [139]. On the other hand, novel mechanisms in the pathogenesis of PPRM suggest the initiation of additional new research. Furthermore, our data has shown that lncRNAs associated with collagen, MMP, proteasome 26S, and ubiquitin specific peptidase were differentially expressed in placentas and amniochorionic membranes of HMV68 viral infected

mice, compared to non-infected, which further indicated that there is a closed link to infection, UPS, collagen and membrane rupture [141]. Therefore, combined with the reviewed data above, a novel molecular pathogenesis of PPRM may be concluded that UPS-related lncRNAs are triggered by infection and inflammation in PPRM, which regulate intracellular and/or extracellular UPS systems to control the content of collagens in ECM, and finally result in occurrence of PPRM for the ECM degradation in fetal membrane.

## Conclusion and Perspective

Growing evidence has suggested that abnormally expressed ncRNAs were involved with multiple diseases. Among various ncRNAs, lncRNAs attracted more attention and were found in correlation with various inflammation-related states or diseases, which might provide new avenues for explaining molecular regulation mechanisms of the complicated diseases on the epigenetic level. Here, we reviewed the UPS system; the collagen in ECM, the PPRM as well as ncRNAs, and hypothesized a novel pathogenic pathway of “infection/inflammation  $\Rightarrow$  lncRNA  $\Rightarrow$  UPS  $\Rightarrow$  collagen  $\Rightarrow$  membrane rupture” for further exploration on the molecular pathogenesis of PPRM.

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