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Mini-Review

Endoplasmic reticulum stress and unfolded protein response in cartilage pathophysiology

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ABSTRACT

After encoding by the nuclear genome, all proteins are translated in the cytoplasmic free ribosomes. While some of them remain in the cytosol, others are targeted into the endoplasmic reticulum (ER). Entry into the ER and folding process must be completed correctly and checked by the quality control system in the ER. ER-resident foldases and molecular chaperones are members of the ER protein quality control system. When the activity in the protein folding system exceeds the capacity of the ER and the quantity of the incorrectly folded proteins increases, the incorrectly folded proteins begin to accumulate in the lumen of the ER. This process triggers ER stress and has a major impact on the pathogenesis of several diseases. Three major ER transmembrane stress transducers trigger adaptive gene expression cascade and restoration of the ER homeostasis. Unfolded protein response (UPR) and endoplasmic reticulum stress have a major impact on both physiological developments of cartilage tissue and pathological situations. New treatment methods can be improved by inhibition of ER stress, activation of chaperon-mediated protein folding or increasing endoplasmic reticulum-associated degradation (ERAD).

KEYWORDS: endoplasmic reticulum stress, unfolded protein response, cartilage tissue, treatment.

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INTRODUCTION

The proper targetting of proteins to their correct final cellular destination is a must to sustain the order and organization in all cells. As reported previously the synthesis of most secretory proteins begins on free ribosomes in the cytosol. Therefore 30% of the proteome are initially destined for the secretory pathway including endoplasmic reticulum (ER) and Golgi apparatus [1, 2].

All proteins encoded by the nuclear genome are synthesized in the cytoplasm; however, some proteins (lysosomal and cell membrane proteins) that need extra lipid and carbohydrate addition, are transferred to ER lumen by an evolutionary-conserved mechanism named as co-translational transport.

Signal-recognition particle (SRP) is a molecule that binds to proteins possessing N-terminal hydrophobic signal sequences on the nascent polypeptide as soon as it emerges from the large subunit of the ribosome. This recognition causes "elongation arrest" and slows down the rate of protein synthesis. Moreover, another function of the SRP is to deliver its cargo to the ER where selectivity is directed by the SRP receptor which is associated with an integral channel protein named as translocon channel. The synthesized proteins are transferred to the ER through these channels. On snipping off the signal sequence by signal peptidase enzyme that is also associated with the translocon channel the halted translation is activated and the growing amino acid chain in an unfolded state slides into the ER lumen.

In comparison to the cytoplasm, the ER lumen not only has a high Ca^{+2} concentration but oxidizing redox potential as well. Both during entry into the ER and/or during the folding process proteins are modified. This includes glycosylation by the oligosaccharyltransferase (OST) complex, disulfide bond formation by protein disulfide isomerases or lipid conjugation. To gain function the proteins have to be folded in the ER from where they are exported to their site of action. However, because of the complex nature of proteins synthesized in the ER, errors in protein folding are unavoidable. So the resulting misfolded/unfolded proteins must be removed to prevent the assembly of defective protein complexes. This instance strongly necessitates a functional protein quality control in the ER [1-6].

2. Molecular mechanisms and chaperons

In light of the recent data, ER-resident foldases and molecular chaperones assist newly synthesized proteins to reach their native structures. Therefore, both of them are considered to be natural members of the ER protein quality control system [4, 7, 8].

Protein disulfide isomerase and peptidyl-prolyl isomerase are the two foldases that catalyze the formation of disulfide bonds and isomerization of peptide bonds proximal to pro-residues, respectively [9].

Recent advances in the survey of protein maturation and quality control processes of the secretory pathway indicate the importance that N-glycans have emerged as key maturation and quality control tags that signal the fate of the protein to which they are attached [10, 11]. Chaperones can be divided into 3 main categories.

2.1. General chaperones

78 kDa glucose-regulated protein (GRP78/BIP), which is a member of this group, is the most abundant chaperone in the ER. Besides GRP78/ BIP, other chaperones like GRP94 and GRP710 are also defined and they participate in the folding dynamics. They capture the hydrophobic locus on the incorrectly folded proteins and thus prevent the formation of aggregates. Also, they facilitate the correct folding of the proteins.

The most known general chaperone is GRP78. This chaperone has an active role in the ER translocon channel, to which the incorrectly folded proteins are directed. Furthermore, they also inactivate the transcription factors (ATF6, XBP1, and IRE1) in the ER membrane, which play a major role in the ER stress response. As GRP78/BIP is a protein that exists in the cytoplasm, nucleus, mitochondria, and plasma membrane and its function changes according to its location, it can be concluded that this protein has more functions than generally assumed [10-13].

2.2. Lectin chaperones

Calnexin and calreticulin are lectin chaperones found in the ER lumen. They are responsible particularly for the quality control of the monoglycolized proteins. The proteins that fail in quality control are directed to endoplasmic reticulum-associated degradation (ERAD) [10, 11].

2.3. Other chaperones

Another feature of the proteins synthesized in the ER is the disulfide bonds, which are formed by the oxydoreductases-like ERO1 found in ER. ERO1 and thioredoxin-like protein disulfide isomerases (PDI) are the major folding proteins in the ER lumen and are responsible for the formation of the S-S-bonds in the proteins. Protein-protein interactions (PPI) and PDIs control the folding process like foldases [10-13].

3. ER stress sensors

The protein folding capacity in the ER lumen depends on the cell type. The ER-protein quality control system (PQCS) is especially developed in the secretory cells. However, if the activity in the protein folding system exceeds the capacity of ER and the quantity of the incorrectly folded proteins increases, PQCS becomes dysfunctional and the incorrectly folded proteins begin to accumulate in the lumen of ER. This process triggers ER stress and has a major impact on the pathogenesis of several diseases such as Type 2 diabetes, obesity, cancer, Parkinson's disease (PD), Alzheimer's disease (AD), osteoarthritis (OA), etc. [14, 15]. By understanding the molecular pathogenic mechanism new pharmacological treatments can be developed. Therefore it is vitally important to understand the defense mechanism of the cell under stress conditions.

The increased metabolic load, infection, hypoxia, exposure to chemical toxins, excessive lipid accumulation, malnutrition and mutations in the protein-coding genes are known as the causes of misfolded protein aggregation. The proteins in the ER can be converted to the aggregation-prone conformation and may trigger the cellular stress at their location. Furthermore, the deterioration of the Ca^{+2} homeostasis impairs the function of the calcium-dependent chaperones like calnexin and calreticulin and increases the ER destruction. The cells try to respond to the ER stress *via* the activation of the sophisticated surveillance system [4, 16].

This response increases the transcription and translation of the gene families related to the restoration of the ER hemostasis. The cells' surveillance may depend both on the duration and severity of the stress. If they cannot adapt, apoptosis is activated, which is also defined as programmed cell death, in order not to harm the surrounding tissue.

The cell uses a specific signal pathway, which is called an unfolded protein response (UPR), to overcome the ER stress. The protein kinase RNA-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1) are the three major ER transmembrane stress transducers. Depending on the increase of the unfolded proteins in the ER lumen, the sensor proteins, which are kept inactive by GRP78 in the ER membrane, are activated. During the activation process, GRP78 is dissociated from the 3 stress sensors to bind to the incorrect protein. The stress sensors with an integral protein structure have a cytolytic domain and can activate the signal effectors. Thus, the adaptive gene expression cascade is initiated from the cytoplasm towards the nucleus. The main target of this cascade is the inhibition of the incorrectly folded proteins and the synthesis of the proteins, which participate in the restoration of the ER homeostasis [4, 17]. (As shown in Figure 1 and Figure 2) [15, 18].

3.1. PERK pathway

PERK is a transmembrane protein having both N-terminal stress-sensing and cytosolic kinase domains. Activated PERK phosphorylates the

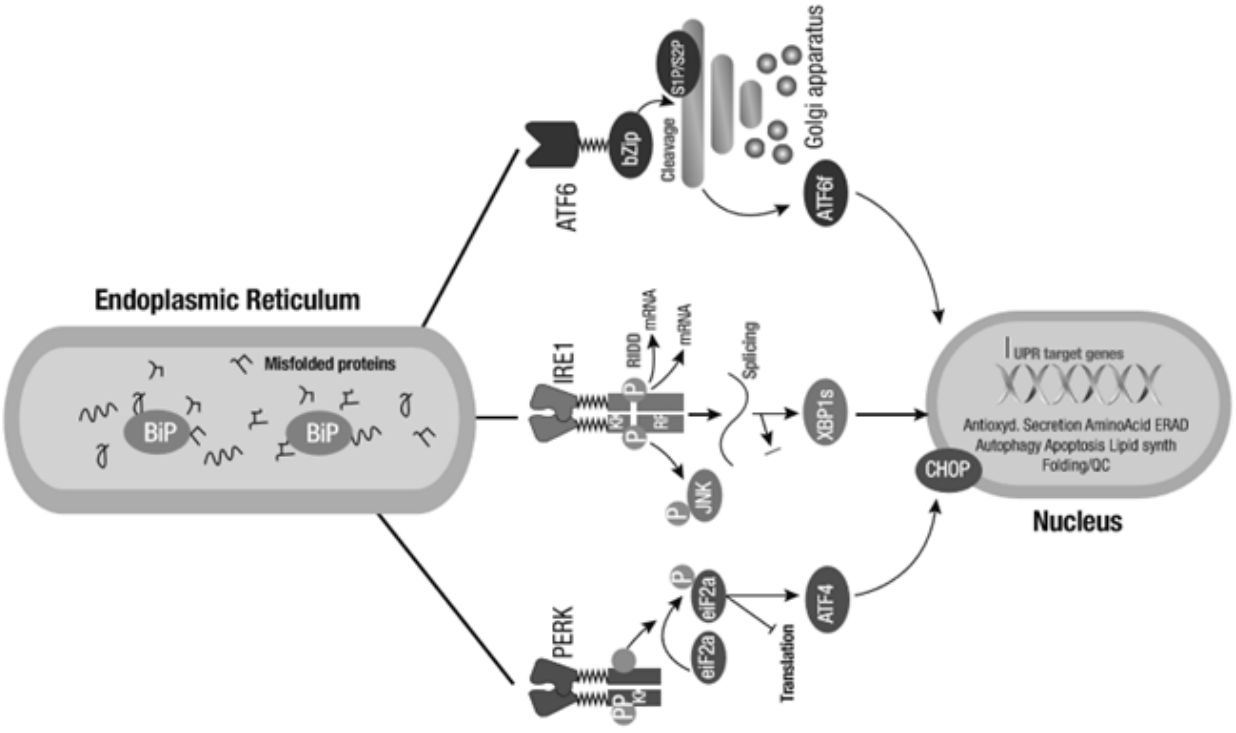

Figure 1. ER stress sensors' signaling pathways and their effects on the nucleus.

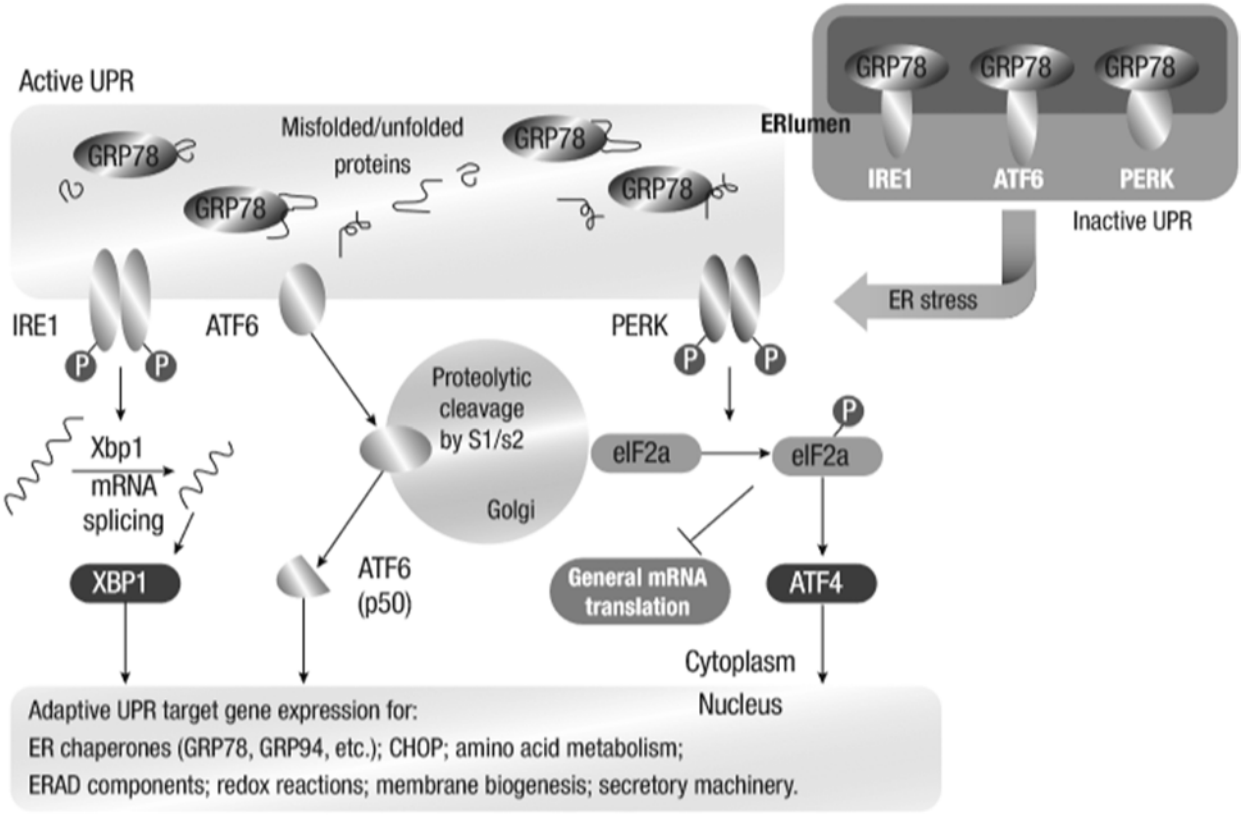

Figure 2. Overview of the three signaling branches of the ER stress response/UPR and its consequences.

α-subunit of the eukaryotic translation initiation factor 2α (eIF2α) at Ser51, leading to rapid and global attenuation of protein synthesis. eIF2-α phosphorylation paradoxically enhances the translation of several mRNAs, including the transcription factor namely activating transcription factor 4 (ATF4) that promotes the expression of its target genes, including the growth arrest and DNA damage-inducible protein 34 (GADD34) to dephosphorylate eIF2α and restore global mRNA translation. Furthermore, ATF4 activation leads to the production of molecular chaperones and ERAD for improving ER protein folding or clear off the misfolded proteins by the ubiquitin-proteasome system.

Moreover, ATF4 activates C/EBP homologous protein (CHOP), which is another molecule playing a key role in ER stress. CHOP may activate the pro-apoptotic proteins and stimulate the survival genes. The direction of the process is determined by miR-211, which is activated by PERK. It controls the expression of CHOP and suppresses the activation of the pro-apoptotic genes [4, 17, 19, 20].

3.2. IRE1-α pathway

IRE1α, the most conserved branch of the UPR, is a type I transmembrane protein where the ER luminal dimerization domain is structurally related to PERK and the cytosolic domain contains dual catalytic functions of a serine/threonine kinase and an endoRNase (RNase). During the ER stress, IRE1-α released from GRP78 dimerizes and becomes active following autophosphorylation. Activation means the increase of the endoribonuclease activity. This endoribonuclease causes intron splicing in the mRNA of XBP1. This causes the activation of the transcription factor called XBP1s. It increases its transcriptional activity and thus triggers the expression of chaperone and foldase. The activated mRNA of IRE1-α degrades mostly mRNA. The aim is to decrease the protein load as much as possible. This event is defined as IRE1α-dependent decay (RIDD).

The activated IRE1α recruits the TNF receptor-related factor 2 (TRAF2) and apoptosis signal-regulated kinase 1 complex. The IRE1a+TRAF2+ ASK1 complex enables the JUNK activation induced by ER. IRE1a and TRFA also enable the activation of caspase 12. Another link between the ER stress and apoptosis is the bondage of the pro-apoptotic proteins like Bax and Bak to the cytolytic domain of IRE1 [20, 21].

3.3. ATF6 pathway

It is transferred to Golgi after the release from the GRP78. In the Golgi, it is cut by proteases and enters the nucleus and increases the expression of GRP78, GRP94, protein disulfide isomerase, CHOP, XBP1, and calreticulin. ATF6 exists in two isoforms, α and β, both of which are ubiquitously expressed and localized in the ER. Although ATF6α and ATF6β possess significant sequence homology, these isoforms exhibit divergent transcriptional activation domains. Indeed, ATF6α is a potent transcriptional activator whereas ATF6β, a poor transcriptional activator, may inhibit activation by ATF6α [22].

ATF6-α is a type II transmembrane protein that contains a cytosolic cAMP-responsive element-binding protein/ATF basic leucine zipper domain. Upon accumulation of misfolded proteins, the released ATF6α traffics to the Golgi complex where it is cleaved by the serine protease site-1 (S1P) and metalloprotease site-2 (S2P) to produce a cytosolic fragment (p50ATF6α), an active transcription factor [20]. p50ATF6α can act independently or synergistically with XBP1s to mediate the adaptive response to ER protein misfolding by increasing the transcription of genes that increase ER capacity and the expression of Xbp1 [19-21].

4. ER Stress and diseases

Understanding the molecular pathways of ER stress and cell death will significantly contribute to the elucidation of the pathology of neurodegenerative diseases, diabetes, metabolic syndromes, and cancer. Moreover, clarification of these mechanisms will enable the development of new treatment strategies.

5. Unfolded protein response (UPR) and endoplasmic reticulum stress in cartilage pathophysiology

Recent reports add the knowledge that the unfolded protein response (UPR) and endoplasmic reticulum stress have a major impact on both the physiological development of cartilage tissue and pathological situations such as chondrodysplasia and osteoarthritis [14, 23, 24]. The improper induction of ER stress and UPR is a cause of disease, whereas limited presence is necessary for normal chondrogenic differentiation and hypertrophic maturation.

Endochondral ossification is the situation where long bones are developed from the hyaline cartilage model during the normal developmental process. In such an ossification, cartilage tissue is formed primarily and subsequently, bony tissue replaces the cartilage model. Following mesenchymal stem cell aggregation, the pre-hypertrophic stage is observed first, and transformation to hypertrophic chondrocytes occurs later. Calcified cartilage matrix vessels are occupied by osteoclasts and osteoblasts and transform into bone matrix and hydroxyapatite. Normal bone tissue development is provided by mineralization and osteoprogenitor cells/osteoblasts [25]. SOX 9 acts as an early regulator, whereas SOX 5 and 6 act as late regulators. Extracellular matrix (ECM) proteins are also important in normal bone tissue [23]. UPR has a physiological role in chondrocyte maturation during endochondral ossification. UPR has been demonstrated to be continuously activated in response to transient and improper folding in chondrogenesis. BMP2, a UPR activator, has been shown to provide growth plaque maturation. Its effect has been demonstrated in both osteoblasts and chondrocytes; however, the mechanism of action is unknown [14].

Chondrodysplasias are genetic disorders that affect cartilage development and growth. Their incidence is approximately 1/4000. Collagen II, XI, and IX form a heterodimer and construct the fibrillary network of the cartilage, which comprises 2/3 of an adult articular cartilage. Mutation in the gene that codes the α chain of fibrillary collagen results in endoplasmic reticulum dilatation, and related UPR activation. Collagen loss from the

extracellular matrix alters structural integrity, and accumulation in the endoplasmic reticulum activates the UPR. The impaired network structure causes a vulnerability to mechanical damage and the development of osteoarthritis, and relatedly, early osteoarthritis has been demonstrated in chondrodystrophy [26].

As a result of the mutations observed in genes coding ECM molecules, mutant ECM proteins lead to both qualitative and quantitative changes in the composition of the matrix. The main effects are accumulation in the endoplasmic reticulum due to improper folding, production, and secretion. Changes in the connections between the matrix components lead to changes in the organization and function [27, 28]. Although the phenotype of the disease changes according to the region of affected cells, it is generally characterized by skeletal deformity, dwarfism, craniofacial deformities, and early osteoarthritis. Mutations observed in type X collagen leads to metaphysical chondrodysplasia type Schmid (MCDS), mutations observed in Matrillin 3 leads to multiple epiphysial dysplasias (MED), mutations observed in COMP leads to MED+PSACH (pseudoachondroplasia), and mutations in type II collagen leads to spondyloepiphysial dysplasia congenita (SEDC) [26]. In general, irregular growth plaque, reduced proliferation, ceased hypertrophic maturation and increased cell death, are observed in chondroplasias as a result of impaired ECM and UPR activation [26-28]. BBF2H7 and ATF4 have been demonstrated to be important ER stress sensors in the development of chondrodysplasia. BBF2H7 is a membrane-bound transcription factor and structurally resembles ATF6. It codes one of the components of Sec23a, COPII (coat protein 2) complex. BBF2H7 increases the transcription of Sec23a. The BBF2H7-Sec23a pathway is important in cartilage formation through COPII vesicles. BBF2H7 deficiency leads to a transportation defect from ER to Golgi through Sec23a. Studies have demonstrated reduced cartilage matrix, reduced proliferation zone size, grown ER and short extremities in BBF2H7 deficient mice [23, 24, 29]. In the case of ER stress, BBF2H7 is transferred from the ER to Golgi and is separated into two parts. N-terminal (includes bZIP domain) acts as a transcription factor in the nucleus and is responsible for transport vesicle formation. C-terminal is secreted into the extracellular space and binds to Ihh (Indian hedgehog) and functions both in chondrocyte proliferation and PTHrP production. Likewise, ATF4 has an important role in chondrocyte proliferation and differentiation. It acts as a transcription factor in chondrocytes for Ihh [23].

Chondrocyte ER homeostasis is important in the development of the cartilage. To a certain degree, autophagia has a role in normal growth plaque homeostasis, whereas excessive amounts lead to chondrodysplasia. In chondrodysplasias, early osteoarthritis is observed due to impaired matrix structure, reduced resistance to mechanical pressure and compressive loads, increased ER stress due to cyclic mechanical load, reduced chondrocyte proliferation and increased apoptosis [26].

It is unknown what the trigger of OA is. The etiology is believed to be multifactorial. Whether chondrocyte apoptosis is a cause or outcome of cartilage degeneration has been investigated. Apoptosis is the common way of chondrocyte death in the articular cartilage in OA. ER stress, oxidative stress, and inflammatory response are related to each other in the development of OA. ER stress triggers apoptosis through UPR (IRE1, PERK, ATF6). ER stress markers such as BiP, phosphorylated PERK/JNK, CHOP, and XBP1 have been observed to be increased in the articular cartilages of patients with OA. Markers such as PERK, CHOP, and pJNK have been demonstrated to be correlated to cartilage degeneration. CHOP, NO (nitric oxide) and MMP-13 activation increase apoptosis. MAPK, NF-κB, and eIF2α are signal pathways effective in the development of OA. In studies investigating the effects of the IRE1/XBP1 signal pathway, it was demonstrated that matrix components such as aggrecan, collagen I and II were decreased, and ADAMTS5, MMP3, and MMP13 were increased with the decrease in XBP1S 9. XBP1S is the inhibitor of ER stress-mediated apoptosis and a negative regulator of apoptosis in osteoarthritis. In another study, XBP1 was demonstrated to be a component of cartilage destruction in osteoarthritis (Chondrocyte proliferation, differentiation, and hypertrophy) while having a regulator role in cartilage maturation and matrix

mineralization in chondrocyte proliferation and endochondral ossification [29-32].

In another study investigating the response of normal and OA chondrocytes to ER stress and the effects of ER stress markers on cartilage metabolism, PERK and IRE1 were determined to have different roles in physiological conditions and increased ER stress. In homeostatic conditions, IRE1 and PERK act as regulatory molecules, whereas PERK has been demonstrated to be reduced in cartilage tissue in case of osteoarthritis, and consequently leads to a decrease in type II collagen and an increase in type I collagen. The authors believed that PERK could be a new target in the treatment of OA [33].

New treatment targets in cartilage pathologies have been defined as inhibition of ER stress, activation of chaperon-mediated protein folding, increasing ERAD, reducing inflammatory regulators, treatment with chemical chaperons, RNAi treatment and reducing the expression of mutant collagen [14].

6. Conclusion

It has been shown that molecular pathways of ER stress and cell death are associated with different kinds of diseases. The limited presence of ER stress and UPR is necessary for normal chondrogenic differentiation and hypertrophic maturation, whereas inappropriate induction may result in cartilage pathology. New treatment methods are expected to be developed with the elucidation of the different molecular mechanisms involved in cartilage pathologies.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

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