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REVIEW

The Role of Endoplasmic Reticulum Stress in Differentiation of Cells of Mesenchymal Origin

Ekaterina Turishcheva[1,a]*, Mariya Vildanova[1], Galina Onishchenko[1], and Elena Smirnova[1]

[1] *Faculty of Biology, Lomonosov Moscow State University, 119991 Moscow, Russia*
[a] *e-mail: kitten-caterina@yandex.ru*

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Abstract—Endoplasmic reticulum (ER) is a multifunctional membrane-enclosed organelle. One of the major ER functions is cotranslational transport and processing of secretory, lysosomal, and transmembrane proteins. Impaired protein processing caused by disturbances in the ER homeostasis results in the ER stress. Restoration of normal ER functioning requires activation of an adaptive mechanism involving cell response to misfolded proteins, the so-called unfolded protein response (UPR). Besides controlling protein folding, UPR plays a key role in other physiological processes, in particular, differentiation of cells of connective, muscle, epithelial, and neural tissues. Cell differentiation is induced by the physiological levels of ER stress, while excessive ER stress suppresses differentiation and can result in cell death. So far, it remains unknown whether UPR activation induces cell differentiation or if UPR is initiated by the upregulated synthesis of secretory proteins during cell differentiation. Cell differentiation is an important stage in the development of multicellular organisms and is tightly controlled. Suppression or excessive activation of this process can lead to the development of various pathologies in an organism. In particular, impairments in the differentiation of connective tissue cells can result in the development of fibrosis, obesity, and osteoporosis. Recently, special attention has been paid to fibrosis as one of the major complications of COVID-19. Therefore, studying the role of UPR in the activation of cell differentiation is of both theoretical and practical interest, as it might result in the identification of molecular targets for selective regulation of cell differentiation stages and as well as the potential to modulate the mechanisms involved in the development of various pathological states.

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INTRODUCTION

Endoplasmic reticulum (ER) is a multifunctional membrane-enclosed organelle. Some of its major functions are cotranslational transport, modification, folding, and processing of secretory, lysosomal, and transmembrane proteins [1], i.e., processes that involve multiple enzymes and ER chaperone proteins [2]. Since error-free synthesis and correct folding are essential for protein activity, ER has a protein quality control system. Misfolded

Abbreviations: ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; BiP, immunoglobulin binding protein; BMP2, bone morphogenetic protein 2; BSP, bone sialoprotein; C/EBPα, CCAAT/enhancer-binding protein α; CHOP, CCAAT/enhancer-binding protein homologous protein; ECM, extracellular matrix; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GRP78, glucose-regulated protein 78 kDa; IRE1, inositol-requiring protein 1; MEF, murine embryonic fibroblasts; MyoD, myoblast determination protein 1; NFATc1, nuclear factor of activated T cells cytoplasmic 1; OCN, osteocalcin; Osx, Osterix transcription factor; 4-PBA, 4-phenylbutyric acid; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PPARγ, peroxisome proliferator-activated receptor γ; Ppp1cc, protein phosphatase 1 catalytic subunit γ; PTH, parathyroid hormone; RANK, receptor activator of nuclear factor NF-κB; RANKL, RANK ligand; Runx2, runt-related transcription factor 2; α-SMA, α-smooth muscle actin; sXBP1, spliced X-box-binding protein 1; TGF-β, transforming growth factor-β; UPR, unfolded protein response; XBP1, X-box-binding protein 1.

* To whom correspondence should be addressed.

proteins are recognized by chaperones, such as immunoglobulin binding protein (BiP), ERdj proteins (ER DnaJ family proteins), and lectins, e.g., OS9 which is highly expressed in osteosarcomas and XTP3-B (XTP3 transactivated gene B precursor). Next, misfolded proteins are retrotranslocated from the ER to the cytoplasm, where they are degraded by the proteasomes [2-4]. This process was named the ER-associated degradation (ERAD) [2].

Normal functioning of the ER requires a balance between the synthesis of unfolded proteins in the ER and the activity of ER chaperones [1]. Various physiological states and pathological factors can disturb this balance, leading to the accumulation of incompletely folded or misfolded proteins, a state that was called the ER stress [5]. ER stress can be induced by the accumulation of unfolded proteins in the ER due to, for example, increased demands for secreted proteins in the secretory cells or disturbed protein folding caused by mutations or chemical agents (dithiothreitol, tunicamycin, thapsigargin, brefeldin A, etc.) [6-10]. Disturbances in calcium homeostasis and redox state of the ER, hypoxia, glucose starvation, ER overload with cholesterol, nutrient deficit, and temperature increase to 40°C can all lead to impaired protein folding and result in ER stress [8-10].

Restoration of normal ER functioning and protein folding in particular in response to ER stress is initiated by the activation of a signaling cascade known as the unfolded protein response (UPR) [2, 11]. UPR is an adaptive mechanism aimed at restoring cell homeostasis and promotion of cell survival [9, 10]. UPR activation affects almost all aspects of the secretory pathway by modulating the intensity of protein synthesis and translocation in the ER, protein folding and maturation, protein quality control, protein translocation along the secretory pathway, and elimination of misfolded proteins via autophagy and ERAD [11].

UPR is initiated by three sensor ER transmembrane proteins: IRE1 (inositol-requiring protein 1), PERK (protein kinase RNA-like endoplasmic reticulum kinase), and ATF6 (activating transcription factor 6) [2]. In the absence of ER stress, the domains of these proteins facing ER lumen (luminal domains) bind BiP, or GRP78 (glucose-regulated protein 78 kDa) that prevents the activation of corresponding sensor proteins. In the case of ER stress, BiP dissociates from the sensor proteins, resulting in their activation [9]. Hence, IRE1, PERK, and ATF6 "monitor" whether the activity of the ER folding machinery is sufficient for the amount of newly synthesized proteins [2].

Each ER sensor protein (IRE1, PERK, or ATF6) initiates its own signaling cascade upon activation. Depending on the stress factor and cell type, ER stress might initiate only one or two of the three signaling cascades [1].

If UPR activation fails to restore normal functioning of the ER, the cell initiates signaling pathways resulting in the cell death [1, 2]. Therefore, chronic ER stress and long-term UPR can lead to the disturbance of cell functioning and cell death [1, 12]. In turn, these processes can be involved in the development of chronic diseases in humans, including neurodegenerative disorders, diabetes, lung fibrosis, and inflammation [12].

Cell death or survival is not the only outcomes of ER stress. Besides its involvement in controlling protein folding, UPR plays a key role in various physiological processes, such as innate immunity, glucose and lipid metabolism, and cell differentiation [11]. Physiological ER stress modulates the differentiation of cells of connective, muscle, epithelial, and neural tissues [13-16]. Thus, physiological ER stress induced by plant hormones (gibberellic and jasmonic acids) activates differentiation of immortalized keratinocytes of the HaCaT line and leads to the appearance of cell differentiation signs in human epidermoid carcinoma A431 cells [17, 18]. Gibberellic and abscisic acids induce morphological signs of the ER stress in the connective tissue cells, such as human skin fibroblasts and human fibrosarcoma HT1080 cells [19]. In this article, we reviewed the data on the role of ER stress and UPR in the differentiation of cells of mesenchymal origin, namely, fibroblasts, preadipocytes, myoblasts, osteoblasts, and osteoclasts.

UPR PATHWAYS

As mentioned above, UPR can be initiated by three transmembrane ER stress sensors – kinases IRE1 and PERK and transcription factor ATF6. During ER stress, chaperone BiP dissociates from the luminal domains of these proteins, resulting in their activation and the induction of three distinct signaling cascades (Fig. 1).

IRE1-activated pathway. Following the dissociation of BiP, the transmembrane kinase IRE1 undergoes oligomerization and autophosphorylation, resulting in its activation [10]. The endoribonuclease domain of the activated IRE1 catalyzes the splicing of mRNA encoding the XBP1 transcription factor. The excision of a 26-nucleotide intron from the mRNA causes a frameshift, leading to the synthesis of active sXBP1 ("s" for spliced) transcription factor [11, 20] that upregulates the expression of proteins involved in the translocation of synthesized protein into the ER, their folding (protein disulfide isomerase, PDI), and secretion, as well as degradation of misfolded proteins (ERAD-enhancing α-mannosidase-like protein, EDEM) and production of ER lipid components (choline cytidylyltransferase) [1, 11, 21, 22].

PERK-activated pathway. After the release of BiP, the transmembrane kinase PERK undergoes oligomerization and autophosphorylation and phosphorylates Ser51 of the α-subunit of eIF2 (eukaryotic translation initiation factor 2) [10, 23]. Phosphorylated eukaryotic translation initiation factor 2α (eIF2α) suppresses translation initiation, leading to the translation inhibition for

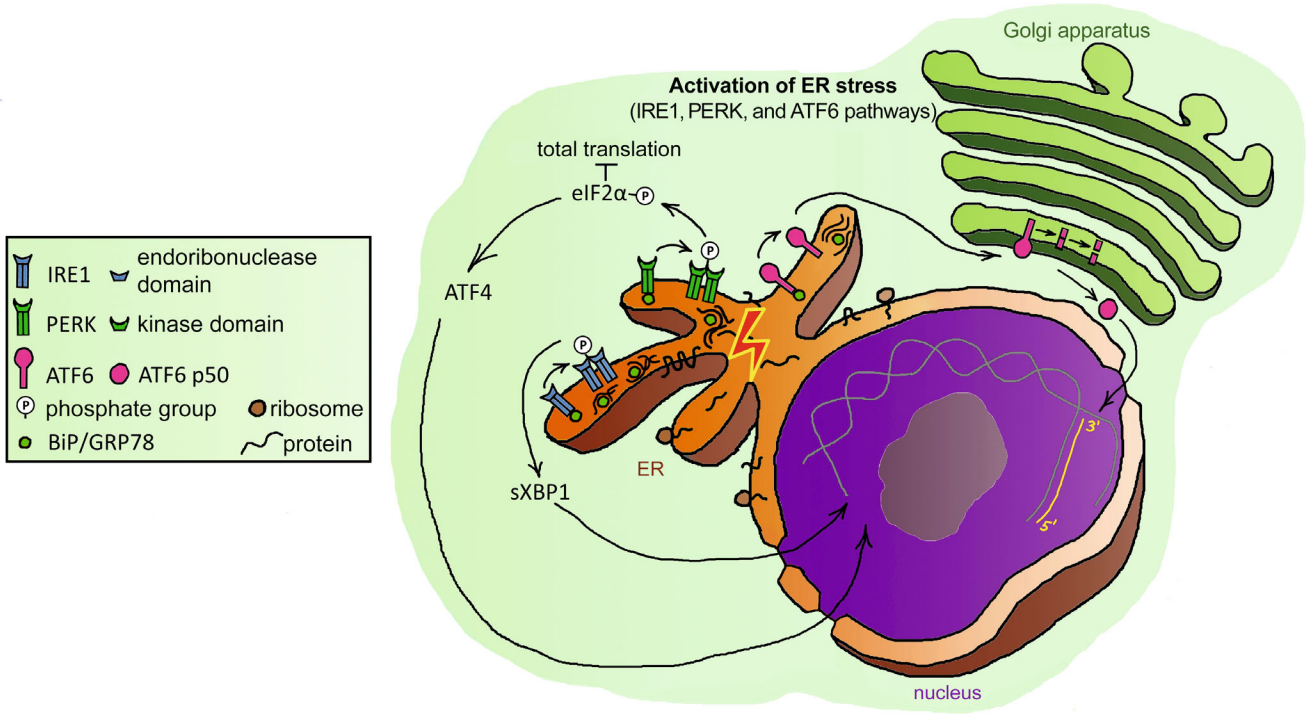

Fig. 1. Key events in the development of ER stress and UPR after dissociation of BiP/GRP78 from the transmembrane ER stress sensors (IRE1, PERK, and ATF6). IRE1 undergoes oligomerization and autophosphorylation; its endoribonuclease domain catalyzes the splicing of mRNA for XBP1 (X-box-binding protein 1), resulting in the synthesis of sXBP1 (spliced X-box-binding protein 1) transcription factor. PERK undergoes oligomerization and autophosphorylation and phosphorylates eIF2α (eukaryotic translation initiation factor 2α), leading to the suppression of total translation in the cell and activation of translation of ATF4 (activating transcription factor 4) involved in the transcription of UPR target genes. ATF6 is translocated to the Golgi apparatus, where it is cleaved by proteases with the release of cytoplasmic ATF6p50 fragment. ATF6p50 is translocated to the nucleus, where it acts as a transcription factor (see green box for the symbols used in the figure).

most mRNAs [23]. As a result, no synthesized proteins are translocated to the ER, lessening the ER load, which is associated with correct protein folding [2]. However, phosphorylated eIF2α upregulates translation of mRNA for the ATF4 (activating transcription factor 4) [11, 23]. ATF4 activates transcription of the UPR target genes coding for the proteins participating in the biosynthesis of amino acids, antioxidant defense, autophagy, and apoptosis [11, 23]. The eIF2α-mediated suppression of translation is reversible, as ATF4 increases the activity of the growth arrest and DNA damage-inducible protein GADD34, a regulatory subunit of protein phosphatase 1 (PP1), thereby participating in a negative feedback loop causing eIF2α dephosphorylation and restoration of normal level of protein synthesis [11].

ATF6-activated pathway. After the dissociation of BiP, the transmembrane protein ATF6 is translocated by COPII-coated vesicles from the ER to the Golgi apparatus, where it is cleaved by S1P and S2P proteases [11, 24] releasing the cytoplasmic fragment ATF6p50. ATF6p50 is then translocated to the nucleus, where it acts as a transcription factor together with sXBP1 [11]. ATF6p50 and sXBP1 activate transcription of genes coding for ER chaperones and enzymes that stimulate protein translocation to the ER, folding (chaperones GRP78, GRP94, and calnexin), processing, and secretion, as well as degradation of misfolded proteins [1, 11, 25, 26]. ATF6p50 and sXBP1 also stimulate biogenesis of the ER and Golgi apparatus during ER stress [11].

Activation of cell death in chronic ER stress. In case of pronounced chronic ER stress, IRE1 initiates the TNF (tumor necrosis factor) signaling pathway by recruiting TRAF2 (TNF receptor-associated factor 2, a mediator of the TNF pathway) and activating ASK1 (apoptosis signal-regulating kinase 1). ASK1 activates JNK (c-Jun N-terminal kinase), which is involved in the induction of apoptosis by regulating the BCL2 (B-cell lymphoma 2) family of proteins [1, 10]. Moreover, ATF4, which participates in the PERK/eIF2α cascade, can activate the expression of the pro-apoptotic CHOP (CCAAT/enhancer-binding protein homologous protein). CHOP regulates the BCL-2 family proteins, DR5 (death receptor 5), and GADD34 (PP1 regulatory subunit) and stimulates stress-mediated induction of apoptosis [11].

Therefore, UPR is an adaptive response aimed at cell survival, which, however, can initiate cell death if the restoration of normal ER functioning fails [1, 2, 9, 10]. Alongside its involvement in the protein folding quality control, UPR plays a key role in physiological processes, such as differentiation of cells of connective, muscle,

epithelial, and neural tissues [11, 13-16]. We reviewed the data on the role of ER stress and UPR in the differentiation of different cells of mesenchymal origin.

UPR AND DIFFERENTIATION OF FIBROBLASTS INTO MYOFIBROBLASTS

Fibroblasts are connective tissue cells that synthesize and secrete components of the extracellular matrix (ECM), such as collagen, fibronectin, elastin, hyaluronic acid and other glycosaminoglycans, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), etc. [14, 27]. Fibroblasts are involved in the ECM formation and remodeling, wound healing, inflammation, and angiogenesis [27]. In the case of tissue damage, fibroblasts move to the site of damage, where they proliferate and differentiate into myofibroblasts [14, 27]. Myofibroblasts combine the characteristics of fibroblasts (e.g., well developed rough ER and Golgi apparatus) and smooth muscle cells (contractile apparatus containing smooth muscle actin, α-SMA). Compared to fibroblasts, myofibroblasts have a higher content of collagen I, fibronectin, and TGF-β (transforming growth factor-β), increased number of focal contacts and stress fibers, higher contractile ability, and decreased migratory activity [14, 28]. Myofibroblasts promote wound healing by restoring ECM as a scaffold for tissue regeneration and by closing the edges of the wound due to their contractile activity [27].

In normal wound healing and restoration of the tissue integrity, myofibroblasts die by apoptosis [14, 28]. If the process of wound healing is disrupted myofibroblasts stay in the location of the damage, followed by the development of fibrosis, which is characterized by excessive ECM formation and unregulated contractile activity of the myofibroblasts [28, 29]. Fibrosis can also develop during chronic inflammation caused by toxic compounds or mechanical damage or as a result of autoimmune response (scleroderma, ulcerative colitis, Crohn disease, and rheumatoid arthritis) [29]. Since the volume and rigidity of the ECM play a key role in structural and functional integrity of body tissues, excessive ECM in fibrosis leads to further development and exacerbation of tissue dysfunction. For example, idiopathic lung fibrosis is characterized by the accumulation of myofibroblasts and ECM remodeling, resulting in impaired lung tissue structure and progressive fibrosis [30]. Almost all body tissues can undergo fibrosis (including liver, heart, and kidneys) [27, 29]. The presence of myofibroblasts was observed in actively contracting granulation tissue and hypertrophic scars, as well as in the contractile tissues such as the palmar fascia in Dupuytren's disease [14]. Based on the above information, studying the mechanisms of fibroblast differentiation into myofibroblasts is essential for discovering new approaches for fibrosis prevention and treatment, as well as for amelioration of disease manifestations. To model fibrosis *in vitro*, researchers often use TGF-β, a multifunctional cytokine that is viewed as a major inducer of fibroblast differentiation into myofibroblasts [14, 28, 31].

The participation of ER stress in the differentiation of fibroblasts isolated from different types of tissues into myofibroblasts has been demonstrated in numerous *in vitro* studies [14, 29, 31, 32]. On the other hand, inhibition of ER stress was found to suppress such differentiation [31, 33, 34].

For instance, it was found that the TGF-β-mediated induction of differentiation of mouse and human lung fibroblasts into myofibroblasts was accompanied by a significant upregulation of GRP78, sXBP1, and ATF6 synthesis [31]. At the same time, the levels of CHOP and phosphorylated eIF2α remained the same, which might be related to the antiapoptotic properties of TGF-β. On the contrary, inhibition of the ER stress by the chemical chaperone 4-phenylbutyric acid (4-PBA) not only abolished the UPR development, but also suppressed expression of genes coding for α-SMA and collagen I (main markers of myofibroblast differentiation). Induction of α-SMA and collagen I synthesis by TGF-β was suppressed by the *GRP78* knockdown, demonstrating the importance of UPR components in cell differentiation [31]. 4-PBA also demonstrated an antifibrotic effect in TGF-β-induced differentiation of rat primary synovial fibroblasts into myofibroblasts [33]. ER stress was activated upon the induction of fibroblast differentiation into myofibroblasts by endothelin 1 and thrombin [32].

Interestingly, 1% extract of cigarette smoke caused the differentiation of human embryonic lung fibroblasts (MRC-5 cell line) into myofibroblasts also through the induction of ER stress [35]. It activated the synthesis of α-SMA, GRP78, IRE1, XBP1, and ATF6. The treatment of fibroblasts with 4-PBA or *GRP78* knockdown prior to the exposure to cigarette smoke extract reduced fibroblast differentiation into myofibroblasts, although it remained unknown whether the effect of cigarette smoke resulted from the action of one or several extract components or if it was produced by the combined action of all components [35].

Experiments on the inhibition of individual UPR pathways has demonstrated their importance in the activation of fibroblast differentiation into myofibroblasts; however, it remains unclear if these pathways are equally important in this process [29, 32, 36].

IRE1 signaling pathway. IRE1 inhibition by the selective inhibitor 4μ8C (salicylic aldehyde derivative) that suppresses its RNase activity, blocked the TGF-β induced differentiation of human embryonic lung fibroblasts into myofibroblasts by downregulating the expression and synthesis of α-SMA and collagen I [29]. Similar decrease in α-SMA gene expression was observed in hu-

man embryonic lung fibroblasts expressing RNase-suppressed mutants of IRE1 and mouse embryonic fibroblasts with *IRE1* gene knockout. Moreover, IRE1 cleaves miRNA-150, which abolishes the inhibitory effect of this microRNA on *α-SMA* expression via suppressing the biosynthesis of c-Myb transcription factor. This mechanism is involved in the downregulation of the TGF-β-induced expression of *α-SMA* upon inhibition of the RNase IRE1 activity. sXBP1 stimulates the synthesis of phosphatidylcholine (major ER membrane lipid), contributing to the increase in the ER area, which is essential for more efficient protein synthesis during fibroblast differentiation into myofibroblasts [29, 37], and knockdown of *XBP1* prevents the TGF-β-induced ER expansion and collagen secretion [29].

PERK signaling pathway. Knockdown of *PERK* suppressed the differentiation of human embryonic lung fibroblasts of the WI-38 cell line into myofibroblasts [32]. In the absence of PERK, induction of cell differentiation by endothelin 1 or thrombin failed to upregulate the synthesis of α-SMA, collagen I, and collagen IV. A JNK inhibitor also suppressed the differentiation of WI-38 fibroblasts into myofibroblasts induced by endothelin 1 or thrombin; in particular, no increase in α-SMA synthesis was observed. This suggests that JNK activates PERK, while PERK is essential for the activation of α-SMA synthesis.

ATF6 signaling pathway. ATF6 prevents the differentiation of heart ventricle fibroblasts into myofibroblasts. Inactivation of ATF6 by knockout or knockdown promoted the profibrotic effect of TGF-β [36]. In contrast, pharmacological activation of ATF6 by low-molecular-weight activator *N*-(2-hydroxy-5-methylphenyl)-3-phenylpropanamide (compound 147) downregulated *α-SMA* expression and suppressed the profibrotic action of TGF-β. It was suggested that ATF6 suppresses fibroblast differentiation into myofibroblasts by inhibiting the TGF-β-mediated signaling via Smad and by decreasing the expression of genes coding for TGF-β and its receptors. In particular, ATF6 activation by compound 147 caused a decrease in the activity of certain profibrotic genes (e.g., genes of metalloproteinases and TGF-β receptors), while *ATF6* knockdown upregulated the expression of those genes [36].

Effect of ER stress inducers on fibroblast differentiation into myofibroblasts. ER stress can be induced by chemical agents, for example tunicamycin and thapsigargin. Tunicamycin is an antibiotic that blocks N-glycosylation of proteins in the ER, resulting in the disruption of the initial stages of glycoprotein biosynthesis, accumulation of unfolded proteins in the ER, and ER stress [1]. Thapsigargin is a sesquiterpene lactone that inhibits Ca^{2+}-ATPase (SERCA) and causes loss of activity of Ca^{2+}-dependent chaperones, accumulation of unfolded proteins, and ER stress. Both tunicamycin and thapsigargin were found to induce fibroblast differentiation into myofibroblasts. Thus, treating human primary lung fibroblasts with tunicamycin or thapsigargin led to the activation of α-SMA and collagen I synthesis, while knockdown of *GRP78* prevented this activation [31]. Thapsigargin-induced ER stress also upregulated the expression of the collagen I and α-SMA genes in rat fibroblasts [33]. Continuous activation of ER stress with tunicamycin in mouse skin primary fibroblasts induced differentiation of these cells into myofibroblasts. The cells started to express *α-SMA*, demonstrated contractile activity, and acquired a more flattened shape [14]. Tunicamycin and thapsigargin also activated α-SMA and collagen IV synthesis and stimulated the differentiation of human embryonic lung fibroblasts (WI-38) into myofibroblasts [32]. Knockdown of *PERK* or PERK inhibition by GSK2606414 suppressed thapsigargin-induced synthesis of α-SMA and collagen IV.

Since signaling pathways involved in the activation of ER stress can produce opposing effects on fibroblast differentiation into myofibroblasts, activation of the IRE1- and PERK-dependent mechanisms can be used for stimulation of cell differentiation, while suppression of differentiation requires modulation of the ATF6 signaling cascade.

UPR AND ADIPOGENESIS

Adipocytes are the main components of adipose tissue. They control cell energy balance by storing triglycerides during the periods of energy excess and their utilization in the case of energy deficit [38]. Adipocytes are metabolically active cells that synthesize and secrete various proteins, including hormones (leptin, adiponectin) and cytokines [39]. Adipocytes differentiate from preadipocytes [38]; the key role in this differentiation (adipogenesis) belongs to the transcription factors C/EBPβ and C/EBPδ (CCAAT/enhancer-binding proteins β and δ) that are activated very early and initiate the expression of PPARγ (peroxisome proliferator-activated receptor γ) and C/EBPα (CCAAT/enhancer-binding protein α). PPARγ and C/EBPα are two essential factors of adipogenesis that positively regulate each other to stimulate and sustain the differentiation status of the cells [40]. It should be noted that preadipocytes undergo significant morphological changes during differentiation. For example, their shape changes from fibroblast-like to cuboid, and they accumulate a large number of lipid droplets [39-41].

A common method for the induction of preadipocyte differentiation in *in vitro* studies of adipogenesis is treating the cells with a special cocktail containing insulin, isobutylmethylxanthine, dexamethasone, and troglitazone. Typical cell lines used as preadipocytes are 3T3-L1 cells and mouse embryonic fibroblasts (MEFs) [39, 40, 42-47].

Adipocyte differentiation *in vitro* and *in vivo* is accompanied by the induction of ER stress [39, 45-47]. In *in vitro* system, the content of phosphorylated eIF2α and sXBP1 increases at an early differentiation stage and then gradually decreases, while the synthesis of CHOP, on the contrary, decreases at the early stages of differentiation and then increases during later stages [39, 45, 46]. Inhibition of the ER stress *in vitro* using chemical chaperones 4-PBA or tauroursodeoxycholic acid (TUDCA) suppresses adipogenesis, which manifests as a decrease in the number of lipid droplets and the absence of changes in cell shape. 4-PBA also decreases the secretion of adiponectin. Pulse addition of 4-PBA blocks lipid accumulation at all differentiation stages, from early (days 0-2) to the late one (days 6-10), which points towards the importance of UPR for adipogenesis at all stages. However, the most pronounced suppression of lipid accumulation upon completion of differentiation was observed when 4-PBA was added at the late adipogenesis stages. In the *in vivo* experiments, adding 4-PBA to drinking water caused a decrease in the weight gain in female C57BL/6 mice, reduced the mass and thickness of fat pads, and decreased the size of adipocytes. 4-PBA also reduced the rate of GRP78 synthesis in the adipose tissue. This *in vivo* effect of 4-PBA is likely related to the fact that 4-PBA suppresses recruitment and differentiation of preadipocytes and prevents hypertrophy of already existing adipocytes [39].

Inhibition of adipogenesis by the suppression of the ER stress was also observed upon the action of nonthermal atmospheric plasma (ionized gas), the clinical application of which has been studied during the last decades. The treatment of 3T3-L1 cells for 4 days with the plasma solution obtained by the authors suppressed adipogenic differentiation of these cells and inhibited ER stress and UPR activation [47].

It has been found that successful adipogenesis requires the functioning of all three UPR activation pathways [40, 43, 44, 48].

IRE1 signaling pathway. It was shown that the early adipogenic factor C/EBPβ directly binds to the XBP1 gene promoter and induces its expression. sXBP1 binds to the promoter of another critically important transcription factor – C/EBPα – and induces its expression. Knockdown of *XBP1* or *IRE1* in MEFs and 3T3-L1 cells resulted in major disruptions of adipogenesis, e.g., the absence of lipid droplet formation in the cells. No hyperphosphorylation of IRE1 occurred during adipogenesis in 3T3-L1 cells, although it was observed upon UPR induction by thapsigargin. Interestingly, the development of obesity in mice was accompanied by the induction of IRE1 hyperphosphorylation. This might be related to the fact that physiological UPR is activated during early adipogenesis and then is maintained at a relatively low level in mature adipocytes, while obesity is associated with UPR activation [40].

However, according to other authors, neither activation, nor deletion of the *IRE1* gene affected differentiation of 3T3-L1 cells [45], which contradicts the results reported in [40].

PERK signaling pathway. The PERK-mediated mechanism of the ER stress induction is also involved in the regulation of lipogenesis program during adipogenesis. Thus, the absence of PERK abolished the activation of expression of the lipogenic enzymes SREBP-1c (sterol regulatory element-binding transcription factor 1c), FAS (fatty acid synthase), and SCD1 (stearoyl-CoA desaturase 1) and suppressed the accumulation of lipid droplets. In the course of stimulation of adipocyte differentiation, PERK demonstrated a lipid kinase activity with diacylglycerol as a substrate [44, 49]. However, the synthesis of CHOP (target of PERK) decreased at the early differentiation stages and increased at the late ones, which indicates a necessity for lowering the activity of PERK at the early stages of adipocyte differentiation [39, 45, 46]. Stimulation of eIF2α phosphorylation in the absence of ER stress reduced the efficiency of adipogenesis in 3T3-L1 cells, while suppression of eIF2α phosphorylation, on the contrary, stimulated adipogenesis both *in vitro* and *in vivo* [45]. Increased production of CHOP in the absence of ER stress also inhibited adipogenesis in 3T3-L1 cells [41, 45]. Inhibition of adipogenesis by CHOP might be related to its ability to form heterodimers with C/EBPα and C/EBPβ and prevent the binding of formed heterodimers to the classical binding sites for C/EBP [41, 50]. In other words, CHOP acts as a negative regulator of adipogenesis [39, 41]. Therefore, it is possible that the decrease in CHOP expression at the early differentiation stage, which has been demonstrated by many authors, is due to the fact that such decrease is required for the functioning of adipogenesis genes of the C/EBP family, the activity of which is suppressed by CHOP.

ATF6 signaling pathway. ATF6 is also important for normal adipogenesis. Knockdown of *ATF6* in mesenchymal stromal C3H10T1/2 cells resulted in suppression of lipid accumulation and significant downregulation of the expression of genes for PPARγ, lipogenic transcription factor SREBP-1c, insulin-sensitive glucose transporter GLUT4, and fatty acid-binding protein aP2. The difference in the *PPARγ* expression in *ATF6*-deficient and control cells became more pronounced during the course of adipogenesis. Besides, stimulation of the expression of the adipogenic early transcription factor C/EBPβ was less pronounced in cells with *ATF6* knockdown [48].

It should be mentioned that some authors reported inhibition of adipocyte differentiation by the ER stress [41, 42, 45, 51]. For example, compound K-7174 (synthetic low-molecular-weight inhibitor of GATA transcription factors used to maintain preadipocytes in a nondifferentiated state) and ER stress inducers (tunicamycin, A23187, and thapsigargin) suppressed adipogenesis of 3T3-L1 cells [42, 45]. Moreover, tunicamycin

and thapsigargin inhibited the differentiation of beige adipocytes, the development of which reduces obesity and promotes thermogenesis by the dissipation of excessive chemical energy through the UCP1 (uncoupling protein 1)-mediated heat generation [51]. Alleviation of the ER stress with chemical chaperones TUDCA or 4-PBA stimulated the differentiation of beige adipocytes. Administration of TUDCA to obese mice resulted in browning of white fat, which might be caused by the *trans*-differentiation of white adipocytes into the beige ones, and significantly slowed down the weight gain [51].

It is possible that the discrepancies in the data on the role of ER stress in adipogenesis are caused by the fact that different levels of ER stress can cause different effects in the cells. Initiation of the ER stress at levels exceeding physiological results in a pronounced UPR, which prevents cells from differentiating, because the resources of these cells are redirected toward survival. In regard of this, some researchers suggest the existence of two types of UPR – acute (pathological) and chronic (physiological) [39, 40, 45, 46]. Acute/pathological UPR develops in response to unfavorable conditions, e.g., hypoxia, hyperglycemia, viral infection, or oxidative or mechanical stress caused by obesity-related adipocyte hypertrophy in the adipose tissue. This type of ER stress can negatively affect the functioning of adipose tissue and promote the development of type 2 diabetes, insulin and leptin resistance, and lipotoxicity. In contrast, chronic/physiological UPR activation is typical for cellular processes such as differentiation and may represent an adaptive mechanism aimed at promoting cell survival and efficient functioning of the ER. Activation of UPR in adipocytes likely adapts the cells to the increased load in the ER [39].

The existence of two types of UPR was confirmed by Longo et al. [46], who demonstrated that the ER stress and UPR were "physiologically" activated during adipogenesis of 3T3-L1 cells, while pathological ER stress caused by the glucotoxic action of glucosamine inhibited their differentiation. Induction of preadipocyte differentiation in the presence of thapsigargin caused a significantly higher activation of UPR than during physiologically induced adipogenesis. This hyperactivation of UPR was accompanied by a reduced expression of adipocyte markers (*C/EBPα*, *PPARγ2*, *FABP4/AP2*) and inhibition of adipogenesis. Similar effect was produced by another ER stress inducer – glucosamine, which activates hexosamine biosynthetic pathway involved in many negative effects of hyperglycemia. Interestingly, the addition of the chemical chaperone 4-PBA (50 μM) together with glucosamine to the differentiation medium caused no suppression of physiological upregulation of the *GRP78* and *CHOP* expression during adipocyte differentiation, but abolished the inhibitory effect of glucosamine on adipogenesis [46]. At the same time, 10-20 mM 4-PBA was found to inhibit adipocyte differentiation [39]. As suggested by Longo et al. [46], this discrepancy was related to the ability of high 4-PBA concentrations to fully suppress physiological UPR required for normal adipocyte differentiation, in particular, for the ER adaptation to the increased protein synthesis in the course of differentiation. At low concentration, this chemical chaperone only inhibited pathological ER stress.

Therefore, all three pathways of the ER stress activation (IRE1-, PERK-, and ATF6-dependent) stimulate adipogenesis, and all of them have to be inhibited to suppress adipogenesis and slow down the development of obesity.

UPR AND MYOGENESIS

Myoblasts are precursors of muscle fibers. During early embryogenesis, myoblasts formed from the committed precursors fuse into multinucleated myotubules that then differentiate into muscle fibers [52]. Committed precursor cells that have not undergone differentiation enter quiescence. At the late embryogenesis stages and during the postnatal development, these cells stay between the basal lamina surrounding the muscle fibers [53] and sarcolemma (muscle fiber plasma membrane) and represent a population of satellite cells (myosatellites) that act as muscle tissue stem cells [52, 54]. During the postnatal period, myogenesis is activated during tissue regeneration after the damage of muscle fibers [55], when satellite cells are activated, proliferate, and either fuse with the existing muscle fibers or form new ones [56]. Proteins involved in the activation of myoblast differentiation and maintenance of their proliferative activity are myoblast differentiation protein 1 (MyoD) and myogenic factor 5 (Myf5) [55]. Induction of differentiation suppresses the proliferative activity of myoblasts and activates the expression of genes coding for myogenin and myocyte-specific enhancer factor 2c (Mef2c), which results in myoblast (myocyte) fusion and terminal differentiation. Myogenesis is accompanied by an increase in ER area and volume, as evidenced by the well-developed ER network in the myotubules [52].

Induction of myogenesis *in vitro* causes differentiation of primary myoblasts into myotubules [54]. It was found that physiological ER stress stimulates myogenesis, and myoblast differentiation requires the functioning of all three UPR activation pathways [15, 52, 55, 57]. Moreover, during myoblast differentiation, the expression of UPR genes is also regulated by the transcription factor TEAD4 (transcriptional enhanced associate domain transcription factor 4) synthesized in the developing skeletal muscles in mouse embryos and required for the normal differentiation of C2C12 myoblasts [57]. In particular, *TEAD4* knockdown reduced the expression of the ER chaperone genes, ERAD genes, *ATF6*, *ATF4*,

and *CHOP* (*CHOP* expression is directly controlled by TEAD4), as well as suppressed expression and splicing of XBP1.

In the course of myoblast differentiation, a temporary decrease in the calcium concentration in ER occurs, which is necessary for myogenesis [15, 55]. Blocking the release of calcium from ER suppresses the UPR during myogenesis and inhibits myogenesis itself. In addition, a decrease in the concentration of calcium in ER in differentiating myoblasts in vitro and in vivo leads to the formation of special structures detected by an ER-tracker. These structures are 1-4 μm in size and consist of cisternae of granular ER rolled into concentric rings. One structure contains 4-10 ER cisternae rolled into a ring, located almost at the same distance from each other. They appear in myoblasts on the third day of differentiation and disappear after myoblast fusion [15]. Similar structures, but with a free space in the center (sometimes filled with organelles), are observed in proliferating myoblasts upon induction of ER stress using calcium ATPase inhibitors, thapsigargin and cyclopiazonic acid. On the contrary, tunicamycin, which does not affect the calcium concentration in ER, does not cause the formation of such structures [15]. However, the physiological significance of these ER conformational changes remains unknown.

IRE1 signaling pathway. IRE1 and XBP1 play an important role in cell survival and expression of genes associated with myoblast differentiation [55]. Knockdown of *IRE1* and *XBP1* in C2C12 myoblasts suppressed myotubule formation and expression of myogenesis genes (*Mef2c*, *MyoD*, and myogenin gene) [52, 55]. It should be noted that *XBP1* is a direct target of myogenin and MyoD [58]. The number of apoptotic cells in a population of cells with the *XBP1* knockdown increases, emphasizing the importance of the IRE1/XBP1 pathway for cell survival (via stimulation of differentiation) [55]. Moreover, by binding to the *CDK5* promoter, sXBP1 participates in the regulation of expression of the cyclin-dependent kinase 5 (CDK5) required for myogenesis. It was suggested that sXBP1 regulates the expression of myogenesis genes by inducing *CDK5* expression [55]. At the same time, sXBP1 overexpression suppresses myogenesis by activating Mist1 (MyoD repressor) [59], resulting in the downregulation of myogenesis markers in myoblasts and disturbed myotubule formation. This may be due to the fact that sXBP1 overexpression mimics an increase in the ER stress level, which initiates the mechanism of differentiation inhibition aimed at the restoration of cell homeostasis, i.e., the functioning of the "ER stress control checkpoint" [55, 59].

Knockout of *XBP1* produced no effect on the regenerative myogenesis involving satellite cells in adult mice [56]. Neither the formation of muscle fibers and myotubules, nor the expression of myogenesis markers were affected. This might be evidence that the myogenic factors contributing to the embryonic myogenesis and myogenesis taking place during muscle regeneration in adult organisms and involving satellite cells are different [55, 56]. It is possible that sXBP1 does not participate in regenerative myogenesis or the absence of this transcription factor is compensated by other proteins [55].

PERK signaling pathway. Early differentiation of myoblasts *in vitro* is controlled by a PERK-mediated mechanism via regulating the expression of microRNAs required for myogenesis [52]. The knockdown of *PERK* in mouse skeletal muscle C2C12 myoblasts suppressed myotubule formation, altered the morphology of these cells, and downregulated the expression and synthesis of MyoD. Pharmacological inhibition of PERK with GSK2606414 (inhibitor of PERK phosphorylation) also resulted in suppression of MyoD synthesis. Moreover, *PERK* knockdown altered the expression of microRNAs required for the maintenance of cell differentiation and cell stemness – expression of many myogenesis-stimulating microRNAs (e.g., miR-128) decreased, while expression of microRNAs supporting cell stemness increased. In addition, it activated signaling pathways associated with cell stemness (Nanog, Myc, and Oct4) and upregulated the expression of myosatellite marker *PAX7* [52].

The PERK/ATF4 signaling cascade also activates microRNAs associated with myogenesis at the early stages of myoblast differentiation [52]. ATF4 binds to the promoter regions and stimulates expression of some microRNAs involved in cell differentiation. ATF4 overexpression leads to myoblast fusion into myotubules even in the absence of differentiation stimulus and increases the level of MyoD synthesis in them.

The key regulator of the UPR signaling pathway is the protein phosphatase 1 catalytic subunit γ (Ppp1cc), whose gene is targeted by miR-128, which, in turn, is regulated by the PERK signaling pathway [52]. miRNA-128 suppresses Ppp1cc that acts as a myogenesis inhibitor essential for the formation of the negative feedback loop regulating the activity of UPR-associated signaling pathways. Inhibition of Ppp1cc results in the ER area increase, cell migration and fusion, and formation of myotubules. The activity of miR-128 is negatively regulated by the RNA-binding protein ARPP21 (cAMP-regulated phosphoprotein 21), which competes with it for the binding to the 3'-untranslated region of the Ppp1cc mRNA. During late myogenesis, ARPP21 suppresses the blocking action of miR-128 on Ppp1cc, thus preventing continuous UPR [52].

Beside embryonic myogenesis, PERK signaling regulates regeneration of skeletal muscles mediated by satellite cells [56]. The knockout of *PERK in vivo* disturbed muscle regeneration, which manifested as suppression of myotubule and muscle fiber formation and downregulation of expression and synthesis of myogenesis markers MyoD and myogenin. Pharmacological inhibition of

PERK with GSK2606414 in satellite cells *in vitro* also inhibited myotubule formation [56].

It should be mentioned that CHOP, on the opposite, suppresses *MyoD* transcription and hinders myoblast differentiation *in vitro* [60]. Expression of *CHOP* was observed in a subpopulation of myoblasts that failed to undergo differentiation after incubation in the differentiation medium. The knockdown of *CHOP* caused earlier and more pronounced differentiation, as well as the increase in the number of nuclei in myotubules, while constitutive *CHOP* expression delayed differentiation and decreased the number of nuclei in myotubules. It was demonstrated that CHOP binds to the regulatory elements in the *MyoD* gene and decreases histone acetylation in these loci. Therefore, CHOP likely prevents premature differentiation of myoblasts [60].

ATF6 signaling pathway. The knockdown of *ATF6* in mouse skeletal C2C12 myoblasts suppressed myotubule formation and inhibited MyoD expression and synthesis [52, 61]. However, the level of *ATF6* expression did not change during regenerative myogenesis involving satellite cells [56]. It appears that the role of different UPR signaling pathways is different in embryonic myogenesis and postnatal regenerative myogenesis, although this subject requires additional research.

Effect of ER stress inducers on myogenesis. Tunicamycin and thapsigargin (ER stress inducers) selectively eliminated C2C12 myoblasts incapable of differentiation, thus allowing more efficient differentiation of survived cells into myotubules [62, 63]. In particular, incubation of myoblasts with tunicamycin or thapsigargin before the induction of differentiation resulted in an increase in the number of nuclei in myotubules and larger myotubule size [62]. Such myotubules formed sarcomeres and contracted, which is rarely observed in *in vitro* systems [62]. Incubation of myoblasts with thapsigargin before the induction of differentiation also upregulated the expression of MyoD and myogenin genes [63]. Moreover, mild thapsigargin-induced ER stress abolished suppression of the C2C12 cell fusion caused by the overexpression of the deubiquitinating enzyme USP19 [64].

Therefore, all three UPR activation pathways are essential for normal embryonic myogenesis, but regenerative myogenesis is activated only by the PERK pathway.

UPR AND OSTEOBLASTOGENESIS

Osteoblasts participate in the formation of bone tissue by synthesizing the ECM [65, 66]. They differentiate from preosteoblasts, which in turn originate from mesenchymal stromal (stem) cells upon their activation by the osteogenesis master regulator Runx2 (runt-related transcription factor 2) [66]. The targets of Runx2 are osteopontin, osteocalcin (OCN), and bone sialoprotein (BSP). Differentiation of preosteoblasts (immature osteoblasts) into mature osteoblasts occurs in three stages: (i) preosteoblasts proliferate and express markers, such as collagen I, osteopontin, etc.; (ii) preosteoblasts exit the cell cycle and start to differentiate into osteoblasts by expressing collagen I and active alkaline phosphatase (markers); (iii) immature osteoblasts mineralize the matrix and express OCN (marker) [66]. Therefore, when immature osteoblasts differentiate into mature cells, they produce a large number of proteins, mainly collagen I and OCN [67-69]. Differentiation of osteoblasts requires multiple factors, the key factors being Osx (Osterix) and BMP2 (bone morphogenetic protein 2) [66, 68]. BMP2 stimulates osteogenesis via activation of mild ER stress with the involvement of IRE1, PERK, and ATF6 [68, 70-72].

IRE1 signaling pathway. The role of the IRE1/XBP1 pathway in the differentiation of osteoblasts still remains ambiguous. According to Tohmonda et al. [73], the IRE1/XBP1 pathway stimulates osteoblast differentiation, with transcription factor Osx (requited for bone formation) being the target of sXBP1 [73]. Thus, stimulation of osteogenic differentiation by BMP2 in MEFs was associated with a significant increase in the sXBP1 content, *Osx* expression, biosynthesis of collagen I and OCN, and activity of alkaline phosphatase. At the same time, in cells with the *IRE1* knockout, the BMP2-induced differentiation caused no increase in the alkaline phosphatase activity and resulted in the lower levels of BSP, collagen I and OCN, i.e., the absence of IRE1 inhibited osteoblast differentiation [73].

The opposite results were reported by Guo et al. [74], who demonstrated that the *IRE1* knockdown stimulated BMP2-induced differentiation of mesenchymal stem cells of the C2C12 line into osteoblasts, while *IRE1* overexpression inhibited cell differentiation [74]. The discrepancy between the obtained data can be explained by the fact that the two groups used different cell lines as a model of osteogenesis, as well as by incomplete inhibition of *IRE1* expression by the knockdown.

PERK signaling pathway. Unlike the IRE1 pathway, the importance of PERK activity for osteoblast differentiation is undoubtable [67, 68, 75]. Mice lacking the *PERK* gene develop severe neonatal osteopenia manifested as a decrease in the thickness of the cortical bone, the extent of matrix mineralization, and volume and thickness of trabeculae [67, 68]. Osteopenia results from the deficit of mature osteoblasts, impairments in the osteoblast differentiation, and disrupted procollagen I production. The knockout of *PERK* in mice and primary mouse osteoblasts downregulated the expression of genes for mature osteoblast markers (alkaline phosphatase, collagen I, OCN, and BSP), delayed the formation of mineralized deposits, and suppressed the expression of *Runx2* and *Osx* [67, 68]. On the contrary, transformation of *PERK*$^{-/-}$ mouse primary osteoblasts with a vector carrying the *ATF4* gene restored alkaline phosphatase activity and matrix mineralization to normal levels [68].

PERK is essential for the activation of ATF4, a critical regulator of osteoblast differentiation [68, 75]. ATF4 is a transcription factor required for cell differentiation and expression of osteoblast-specific genes [75]. ATF4 also participates in post-transcriptional regulation of collagen I synthesis. Mice deficient in *ATF4* develop severe osteopenia; in particular, they demonstrate delayed bone formation and mineralization in the embryonic period and decreased number and thickness of trabeculae during postnatal development [75]. The targets of ATF4 are markers of osteoblast differentiation OCN and BSP [68, 75]. ATF4 activates OCN synthesis by forming a complex with Runx2 on the *OCN* promoter [68, 76]. It should be noted that Runx2 is essential for the synthesis of ATF4 itself; the lack of *Runx2* expression suppresses *ATF4* expression [75].

ATF4 activates transcription not only of the OCN gene, but also of the gene for the osteogenesis transcription factor Osx [77]. ATF4 promotes *Osx* transcription by binding to its promoter. The knockout of *ATF4* significantly decreases the synthesis of Osx, although the level of Runx2 required for *Osx* expression does not change.

Interestingly, the parathyroid hormone (PTH), which is commonly used in clinical medicine for the treatment of age-related osteoporosis, modulates PERK signaling and stimulates bone formation [69, 77]. PTH activated PERK in MC3T3-E1 preosteoblasts and primary calvarial osteoblasts and stimulated the activation of marker genes of osteoblast differentiation (*Runx2*, *OCN*, genes coding for alkaline phosphatase and collagen I) [69]. Suppression of the PERK or ATF4 activity with inhibitors or by a knockdown downregulated the expression of marker genes and decreased the activity of alkaline phosphatase, the extent of matrix mineralization, and OCN secretion in these cells. On the contrary, stimulation of eIF2α phosphorylation by salubrinal promoted PTH-induced osteoblast differentiation. Moreover, PTH strengthened the interaction between the chaperone HSP90 (heat shock protein 90) and PERK, while inhibition of HSP90 decreased the synthesis of PERK and suppressed osteoblast differentiation induced by PTH. Therefore, HSP90 is also required for osteoblast differentiation, as it promotes PERK stability [69]. The role of PERK signaling in the PTH-induced activation was confirmed in *in vivo* experiments. In *ATF4*-deficient mice, PTH either failed to induce expression of osteoblast differentiation markers or induced it to a very low extent [77].

It should be noted that PERK stimulates osteogenic differentiation not only in osteoblasts, but also in other cells of mesenchymal origin. When osteogenic differentiation was induced by a standard differentiation medium (α-MEM supplemented with 10% FBS, 100 μM L-ascorbate-2-phosphate, 10 mM β-glycerophosphate, and 10 mM dexamethasone), stem cells of human periodontal ligament (dense connective tissue that attaches the tooth to the alveolar bone) differentiated into osteoblast-like cells with increased expression of osteogenic differentiation markers [78]. Overexpression of *PERK* upregulated the expression of marker genes to an even higher extent and increased the activity of alkaline phosphatase, while PERK suppression inhibited cell differentiation.

ATF4 also plays a key role in the induction of differentiation of bone marrow mesenchymal stem cells into osteoblasts, in particular, by stimulating the synthesis of β-catenin required for cell differentiation [79]. Inhibition of ATF4 expression prevented cell differentiation into osteoblasts. The knockdown of *ATF4* decreased the level of β-catenin protein synthesis, but did not affect the expression of the corresponding gene, which indicates an existence of a post-transcriptional regulation of β-catenin content. ATF4 induced the emergence of osteogenic differentiation features in NIH3T3 fibroblasts, C2C12 myoblasts, and S194 lymphoblasts [65].

ATF6 signaling pathway. Osteogenesis induced by the differentiation factor BMP2 also involves ATF6 [71]. BMP2 stimulates the expression and activation of ATF6 in MC3T3-E1 cells by strengthening Runx2 binding to the *ATF6* promoter. The knockout of *Runx2* abolished BMP2-dependent activation of ATF6. In its turn, *ATF6* overexpression activated the *OCN* promoter, while inhibition of the ATF6 activity blocked the induction of *OCN* expression. It is possible that BMP2 induces osteoblast differentiation via the Runx2-dependent expression of ATF6, which directly regulates the transcription of the *OCN* gene [71]. Interestingly, ATF6 homolog OASIS (old astrocyte specifically induced substance) expressed in bone cells does not affect the expression of *OCN*, but acts as a transcriptional activator of collagen I gene and has a binding site in its promoter [70]. Mice deficient in the *OASIS* gene develop severe osteopenia due to the delayed osteoblast differentiation that is characterized, in particular, by a decrease in collagen I deposition.

Effect of ER stress inducers on osteogenesis. ER stress inducers stimulate osteogenesis in the case of physiological ER stress [80, 81]. Thus, thapsigargin upregulated the synthesis of OCN and BSP in primary mouse osteoblasts and rat bone marrow mesenchymal stem cells; however, this effect was abolished by the knockout or knockdown of *PERK*, respectively [68, 81].

At the same time, excessive (pathological) ER stress suppresses osteogenesis and induces apoptosis [80-82]. For example, thapsigargin stimulated osteogenesis in mouse bone marrow stromal ST2 cells [82], but higher doses of this compound inhibited osteogenesis [81].

Therefore, all three UPR activation pathways stimulate osteogenesis, which can be used for promoting the regeneration of bone tissue.

UPR AND OSTEOCLASTOGENESIS

Osteoclasts are tissue-specific multinucleated macrophages that differentiate from the monocyte/macro-

phage precursors on or near the bone surface [83]. They play an important role in bone resorption and remodeling, and their activity is tightly regulated by hormones and cytokines in order to provide a balance between bone resorption and formation [84]. The shift in this balance toward excessive bone resorption leads to the development of various pathologies, such as osteoporosis, bone metastasis, bone deterioration in arthritis, and periodontitis [84, 85]. Differentiation of osteoclasts is induced by the binding of RANK (receptor activator of nuclear factor NF-κB) expressed on the surface of osteoclast precursor cells to RANKL (RANK ligand) located on the surface of osteoblasts and osteocytes [83, 84]. After binding the ligand, RANK activates various signaling pathways and induces oscillations in the intracellular calcium concentration, leading to the activation of osteoclastogenesis transcription factors, such as protooncogene c-Fos and NFATc1 (nuclear factor of activated T cells cytoplasmic 1) [84]. Recent studies have demonstrated that the UPR signaling pathways, namely IRE1/XBP1 and PERK/eIF2 branches, play an important role in osteoclast differentiation.

IRE1 signaling pathway. When osteoclastogenesis was induced *in vitro* with recombinant RANKL and mouse colony-stimulating factor 1 (CSF1), the IRE1/XBP1 pathway was activated at the early stages of differentiation, reached its peak on the second day after induction of differentiation, and then was inactivated [84]. Inhibition of IRE1/XBP1 significantly suppressed osteoclastogenesis, which led to the decrease in the number of osteoclasts with a simultaneous increase in the number of osteoblasts and bone mass *in vivo*. This is related to the fact that sXBP1 acts as a transcription factor for NFATc1 (master regulator of osteoclastogenesis) and stimulates its transcription by directly binding to the *NFATc1* promoter. Inhibition of IRE1/XBP1 results in the suppression of *NFATc1* transcription and osteoclastogenesis. Note that IRE1 suppression does not affect the expression of other transcription factors involved in the osteoclast differentiation [84]. It was suggested that during osteoclastogenesis, the IRE1/XBP1 pathway is activated due to the development of physiological ER stress that is indirectly caused by oscillations in the calcium concentration in response to RANKL/RANK signaling. In turn, activation of IRE1/XBP1 stimulated *NFATc1* transcription [84].

It should be mentioned that extracellular vesicles released by the multiple myeloma cells induced osteoclastogenesis in the bone tissue via activation of the IRE1/XBP1 cascade, resulting in bone pathologies observed in over 70% patients with multiple myeloma [86]. The cargo of these extracellular vesicles is represented by UPR signaling molecules (e.g., GRP94 and GRP78); engulfment of these vesicles by mouse Raw264.7 macrophages rapidly initiated phosphorylation of IRE1, splicing of XBP1, and activation of *NFATc1* transcription. Therefore, suppression of the IRE1/XBP1 pathway in mouse macrophages with the selective IRE1 inhibitor GSK2850163 upon simultaneous addition to the cultural medium of extracellular vesicles from the multiple myeloma cells inhibits *NFATc1* expression and prevents macrophage differentiation into osteoclasts, as well as suppressing the resorption activity of such macrophages [86].

PERK signaling pathway. RANKL-induced osteoclastogenesis is accompanied by the activation of the PERK pathway. Inhibition of PERK with GSK2606414 suppressed osteoclast formation and their resorption activity, in a mouse model of osteoporosis [85]. Moreover, ATF4 is a transcriptional activator of *NFATC1*. Osteoclastogenesis was suppressed by the *ATF4* knockout both *in vitro* and *in vivo* and can be promoted by the *ATF4* overexpression [87]. It was suggested that physiological ER stress resulting in osteoclastogenesis stimulation, or, more precisely, activation of PERK, is induced by the oxidative stress [85].

Effect of ER stress inducers on osteoclastogenesis. The involvement of ER stress in osteoclastogenesis has led to the idea that differentiation of osteoclasts can be stimulated with ER stress inducers. Indeed, it was demonstrated that thapsigargin (ER stress inducer) initiated osteoclastogenesis by activating the expression and synthesis of NFATc1 and osteoclast markers [88, 89]. Chemical chaperone and inhibitor of ER stress 4-PBA suppressed osteoclastogenesis and NFATc1 activation both in the presence and absence of thapsigargin [89]. Moreover, 4-PBA suppressed osteoclastogenesis induced by polyethylene particles (average diameter, 65 nm) that are also known to activate ER stress [90]. Tacrolimus (immunosuppressor blocking activation of T cells in rheumatoid arthritis) prevented thapsigargin-induced ER stress and osteoclastogenesis by downregulating expression of *NFATc1* [91]. Another ER stress inducer, tunicamycin, also stimulated osteoclastogenesis and induced expression of osteoclast markers [88].

It should be noted that thapsigargin at the concentrations of 0.05 and 0.1 nM stimulated osteoclastogenesis, but produced no effect at a concentration of 0.2 nM [85], indicating that stimulation of osteoclastogenesis takes place only at certain levels of ER stress. This suggestion was confirmed by the data on the effect of salubrinal on osteoclast differentiation [92]. Salubrinal promoted phosphorylation, and therefore, inhibition of eIF2α, resulting in the decrease in total synthesis of proteins (including NFATc1) and suppression of osteoclast differentiation from bone marrow cells in mice [92, 93]. Salubrinal also alleviated the manifestations of osteoporosis in the mouse model of this disease [93]. It is possible that salubrinal induces too strong of the ER stress, that instead of stimulation, results in suppression of cell differentiation.

Therefore, IRE1 and PERK signaling pathways stimulate osteoclastogenesis; hence, both of them should be inhibited to suppress this process.

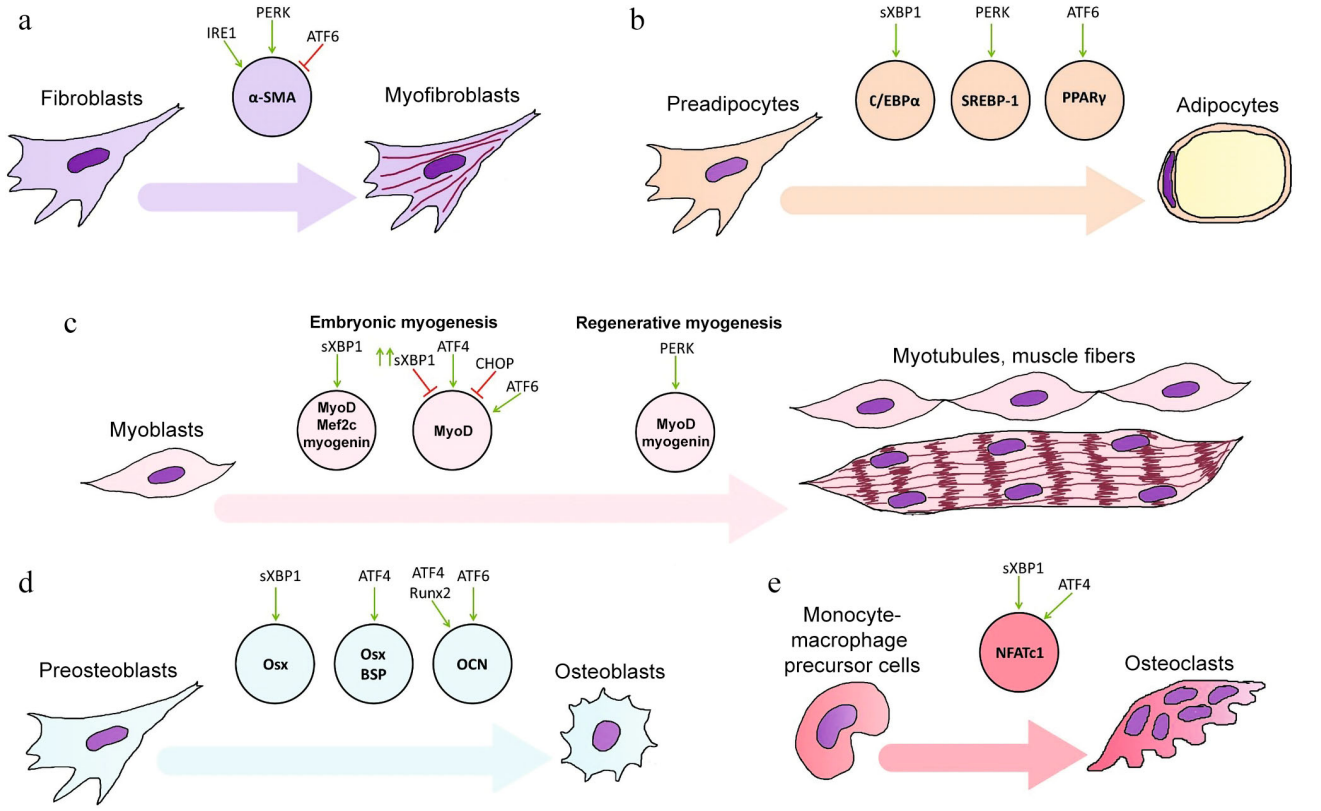

Fig. 2. The role of ER stress mechanisms (IRE1, PERK, ATF6) in the differentiation of cells of mesenchymal origin. Differentiation of fibroblasts, preadipocytes, myoblasts, preosteoblasts, and monocyte/macrophage precursors into mature cells is accompanied by the activation of ER stress. Arrows show stimulation (green) or suppression (red) of synthesis of differentiation markers by the ER stress sensors or components of the UPR signaling cascades. Differentiation markers for each type of cells are shown in the circles of the corresponding color. a) Differentiation of fibroblasts into myofibroblasts; b) differentiation of preadipocytes into adipocytes; c) differentiation of myoblasts into myotubules and muscle fibers (see the differences in the embryonic and regenerative myogenesis); d) differentiation of preosteoblasts into osteoblasts; e) differentiation of monocyte/macrophage precursors into osteoclasts. Designations: ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; BSP, bone sialoprotein; C/EBPα, CCAAT/enhancer-binding protein α; CHOP, CCAAT/enhancer-binding protein homologous protein; IRE1, inositol-requiring protein 1; Mef2c, myocyte-specific enhancer factor 2c; Myf5, myogenic factor 5; MyoD, myoblast determination protein 1; NFATc1, nuclear factor of activated T cells cytoplasmic 1; OCN, osteocalcin; Osx, Osterix transcription factor; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PPARγ, peroxisome proliferator-activated receptor γ; Runx2, runt-related transcription factor 2; α-SMA, α-smooth muscle actin; SREBP1, sterol regulatory element-binding transcription factor 1; sXBP1, spliced X-box-binding protein 1.

CONCLUSIONS

Activation of ER stress and UPR is a requirement for fibroblast differentiation into myofibroblasts, as well as for cell differentiation in adipogenesis, myogenesis, osteoblastogenesis, and osteoclastogenesis (Fig. 2). It appears that it is chronic/physiological ER stress that stimulates differentiation of these cells, while acute/pathological ER stress suppresses cell differentiation and can initiate cell death. As seen in Fig. 2, all three UPR signaling pathways stimulate adipogenesis, embryonic myogenesis, and osteoclastogenesis. Differentiation of fibroblasts into myofibroblasts is activated by the IRE1 and PERK pathways and is suppressed by ATF6 signaling. The PERK pathway (but not the other two signaling cascades) is involved in regenerative myogenesis, which indicates the differences in the regulation of myogenesis in the embryonic and postnatal periods, as well as a potential to control muscle tissue regeneration via regulation of the activity of PERK as an ER stress sensor. Interestingly, some UPR components are transcription factors activating expression of differentiation markers. For example, sXBP1 binds to the promoters of genes for the adipogenesis transcription factor C/EBPα and osteoblastogenesis factor Osx; ATF4 is a transcription factor for the *Osx* and osteoblastogenesis factor *OCN* genes. It remains unclear whether UPR induces differentiation of cells of mesenchymal origin or if it is induced during differentiation, e.g., due to the upregulated synthesis of secretory proteins. Note that activation or suppression of cell differentiation can play a critical role by contributing to the development of various diseases. Thus, excessive differentiation of fibroblasts, adipocytes, and osteoclasts can cause fibrosis, obesity, and osteoporosis, respectively. At present, fibrosis is of particular interest as one of the main negative consequences of COVID-19. Studying

the involvement of UPR mechanisms in cell differentiation and searching for ways of controlling the UPR signaling pathways open new prospects in the development of medicines that would allow to fine-tune or even reroute the processes disrupted by the impairments in the organism development and during the emergence of pathologies.

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