The unfolded protein response and cellular senescence. A Review in the Theme: Cellular Mechanisms of Endoplasmic Reticulum Stress Signaling in Health and Disease

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Pluquet O, Pourtier A, Abbadie C. The unfolded protein response and cellular senescence. A Review in the Theme: Cellular Mechanisms of Endoplasmic Reticulum Stress Signaling in Health and Disease. Am J Physiol Cell Physiol 308: C415–C425, 2015. First published December 24, 2014; doi:10.1152/ajpcell.00334.2014.—The endoplasmic reticulum (ER) is a multifunctional organelle critical for the proper folding and assembly of secreted and transmembrane proteins. Perturbations of ER functions cause ER stress, which activates a coordinated system of transcriptional and translational controls called the unfolded protein response (UPR), to cope with accumulation of misfolded proteins and proteotoxicity. It results in ER homeostasis restoration or in cell death. Senescence is a complex cell phenotype induced by several stresses such as telomere attrition, DNA damage, oxidative stress, and activation of some oncogenes. It is mainly characterized by a cell enlargement, a permanent cell-cycle arrest, and the production of a secretome enriched in proinflammatory cytokines and components of the extracellular matrix. Senescent cells accumulate with age in tissues and are suspected to play a role in age-associated diseases. Since senescence is a stress response, the question arises of whether an ER stress could occur concomitantly with senescence and participate in the onset or maintenance of the senescent features. Here, we described the interconnections between the UPR signaling and the different aspects of the cellular senescence programs and discuss the implication of UPR modulations in this context.

endoplasmic reticulum stress; unfolded protein response; signaling; IRE1α; ATF6α; PERK; senescence; oxidative stress

Functions of the Endoplasmic Reticulum in the Secretory Pathway

THE ENDOPLASMIC RETICULUM (ER) is a membranous tubular network specific of eukaryotic cells that plays a major role in calcium homeostasis, lipid, and protein biosynthesis. It is the first compartment of the so-called protein secretory pathway involved in the biosynthesis and processing of the majority of the secreted proteins, plasma membrane proteins, and membrane or soluble proteins of the different organelles of the secretory pathway itself (i.e., ER, Golgi apparatus, lysosomes, endosomes, and secretory vesicles). About one-third of the genome products follows this secretory pathway (54). After having been synthesized, folded, and assembled in the ER, proteins gain the Golgi apparatus, from which they are addressed to their final compartment.

The ER contains an elaborated protein folding and quality control system that resides in a highly oxidizing environment and a panel of specific ER resident proteins. The newly synthesized proteins entering the lumen of the ER undergo folding, posttranslational modifications, eventually acquisition of disulfide bonds, and eventually assembly in oligomers (54). Protein folding, disulfide bond formation, and oligomerization are facilitated by the oxidizing state of the ER lumen and involve protein disulfide isomerases, peptidyl prolyl cis-trans isomerases, and several chaperones such as BiP/GRP78 or GRP94 (122, 123). Proteins entering the lumen of the ER also undergo an en bloc transfer of a preformed oligosaccharide. This standard oligosaccharide is the matrix of further modifications occurring in the Golgi and giving rise to the final glycoprotein. This initial oligosaccharide is also recognized by the calcium-dependent calnexin and calreticulin chaperones. This interaction permits the retention of the protein inside the ER till it is properly folded (49).

ER Stress

For several reasons including mutations, inadequate stoichiometric amounts of oligomerization partners, shortage of chaperone availability, increase in nascent client proteins, nutrient deprivation, viral infection, hypoxia, and oxidative stress, unfolded or misfolded proteins can accumulate in the ER lumen, aggregate, and hence become toxic and detrimental to cell
survival. Facing these situations collectively referred as ER stress, cells have evolved systems to detect, eliminate, and avoid further accumulation of unfolded or misfolded proteins. One of these systems is the ER-associated protein degradation (ERAD), which exports the unfolded proteins from the ER back into the cytosol where they are degraded by the ubiquitin-proteasome system, for review see Ruggiano et al. (104). Another is the unfolded protein response (UPR). It is by essence an adaptive mechanism that tends to restore ER protein homeostasis (also called proteostasis). It mainly operates by attenuating protein synthesis and by activating a cascade of transcription factors that regulate genes encoding for chaperones, components of the ERAD system, and components of the autophagy machinery. In addition to its function in restoring proteostasis, the UPR controls other pathways of the lipid and energy metabolisms (Fig. 1) (21, 101, 109). When adaptation of cells through the UPR is unsuccessful, due to prolonged or unresolved ER stress, new signals are transmitted from the ER to induce cell death (42, 117). Deregulation of ER homeostasis has been correlated with various physiological and pathological conditions and are well documented in several reviews (14, 25, 69, 82, 106, 134). The ER dysfunction can occur at several levels including ER protein expression levels, ER protein activities, or posttranslational modifications (79, 82, 83, 98).

The Unfolded Protein Response

The UPR involves at least three ER membrane resident proteins: 1) the PKR-like ER kinase (PERK), which through the phosphorylation of the translation initiation factor eIF2α reduces the global synthesis of proteins but enhances the translation of mRNA containing microRNA or IREs including those encoding the ATF4 transcription factor and the chaperone BiP/GRP78 (109). ATF4 controls the expression of many genes involved in redox control and amino acid metabolism, which can lead to both protective and apoptotic signaling pathways (46, 55). The PERK kinase can also phosphorylate the NRF2 protein, which translocates into the nucleus and activates the transcription of genes that maintain the redox homeostasis (31); 2) IRE1α, which, once activated through oligomerization and trans-autophosphorylation, gains endoribonuclease activity and induces the unconventional splicing of XBP1 mRNA to generate XBP1s (135). XBP1s is translated in an active transcription factor, whose main targets are the genes involved in the quality control of proteins in the ER, ER expansion, export, and degradation of misfolded proteins (16, 63, 135). Other mRNAs encoding nonsecreted and secreted proteins are cleaved by IRE1α, via a process called “regulated IRE1-dependent decay” (RIDD); however, the physiological consequences associated with this mechanism are not yet fully known (51, 52, 96). IRE1α can also signal independently of its endoribonuclease activity. Indeed, it has been shown that IRE1α interacts with the adapter TRAF2 to facilitate apoptosis by recruiting and activating ASK1 and JNK (117, 124); and 3) ATF6α is a 90-kDa protein, which, upon activation, exits the ER and migrates to integrate the Golgi apparatus membrane, where it is cleaved by the proteases S1P and S2P. This

![Fig. 1. Activation of the three arms of the unfolded protein response (UPR) in response to endoplasmic reticulum (ER) stress. ER stress triggers the UPR via the activation of the 3 ER transmembrane proteins PKR-like ER kinase (PERK), IRE1α, and ATF6α. These sensors drive specific, coordinated and common responses through the regulation of several transcription factors such as ATF4, XBP1s, and ATF6α-p50 (see text for details). ERAD, ER-associated protein degradation; RIDD, regulated IRE1-dependent decay.](image-url)
cleavage releases a cytosolic 50-kDa domain, which translocates to the nucleus, where it acts as a transcription factor targeting genes encoding mainly quality control proteins including GRP94, BiP/GRP78, PDI, and also other targets such as XBP1 and CHOP (109, 131).

It is now well established that besides its involvement in ER stress response, the UPR allows cells to cope with a high demand of protein load by remodeling and expanding the ER membranes. The requirement for ER expansion has been well documented during the plasma cell differentiation, and several lines of evidence showed that UPR is induced as an anticipated event before the huge antibodies secretion (13, 40). XBP1 and ATF6α play an essential role during B-cell differentiation unlike the PERK arm, which is silenced in this process (41, 140). Indeed, production and secretion of antibodies were markedly impaired in XBP1-deficient B cells or in the presence of a dominant negative ATF6α in response to LPS treatment (44, 100). XBP1s and ATF6α, but not ATF4, were shown to enhance the ER capacity by expanding the tubular networks of rough ER (10, 111, 115). They operate by increasing phospholipid synthesis (10, 115). It is known that the ER is capable of expanding not only in response to lumen demand but also when an increase in resident membrane proteins occurs (67). This again involves ATF6α but not its classical UPR transduction pathway (67). ER expansion involving the UPR can also occur in pathological conditions. XBP1 was shown to promote ER expansion in human bronchial epithelial cells infected with the pathogen Pseudomonas aeruginosa (73). Pathological elevation of fatty acids induced ER expansion, which was reversed by blocking the PERK branch via the chemical chaperone 4-phenyl-3-butenoic acid (4-PBA) (94). Viral infection is also accompanied by complex metabolic changes (including upregulation of the oxidative mitochondria metabolism) (105). Compared with growing cells, senescent cells do not respond to mitotic stimuli or to apoptosis inducers (22, 127). The characterization of the senescent-associated secretory phenotype (SASP) showed an enrichment in proinflammatory cytokines, growth factors, extracellular matrix components, and remodeling enzymes of the extracellular matrix (28).

At least, four types of stimuli can induce senescence. They engage some common but also some specific effector pathways and result in senescent phenotypes, which, consequently, share common and specific markers (Fig. 2). The first characterized type of senescence is replicative senescence. When put in culture after explantation from tissues, normal cells have a limited proliferative potential, the proliferative phase (exponential growth) progressively switching towards a growth stationary phase, which was initially described by Hayflick (48). This replicative senescence phenotype is mainly governed by telomere attrition. The second type of senescence is stress-induced premature senescence (SIPS). It operates independently of telomere attrition and is induced by oxidative stress as well as by many pharmacological drugs or small synthetic and natural compounds (15, 61, 91, 121). Oncogene-induced cellular senescence (OIS) is the most recently described type of senescence. It is observed following the expression of an activated oncogene in normal cells. Several oncogenes have been reported to induce OIS, including H-RasV12 (110), BRAF (77), and NF-κB (9). It is now widely accepted that after an initial step of proliferation, the oncogenic stress drives a

The Senescent Phenotypes

Senescence could be defined as a cellular state characterized by specific genetic, epigenetic, metabolic, and morphological alterations culminating in an irreversible cell-cycle arrest. These changes rely on profound modifications of their transcriptome, proteome, and secretome (6, 7, 28, 112). The overall morphology of the cell is altered at senescence, with an increase in cell size and a change in cell shape. Nuclei and nucleoli are often bigger than in proliferating cells, and senescent cells are frequently polynucleated (30, 143). Cells undergoing senescence also display accumulation of oxidatively damaged proteins (formation of carbonyls) leading to proteotoxicity. They also display changes in the chromatin organization with the appearance of senescence-associated heterochromatin foci (SAHF) (84, 105, 133). Senescence is also accompanied by complex metabolic changes (including upregulation of the oxidative mitochondria metabolism) (105). Compared with growing cells, senescent cells do not respond to mitotic stimuli or to apoptosis inducers (22, 127). The characterization of the senescent-associated secretory phenotype (SASP) showed an enrichment in proinflammatory cytokines, growth factors, extracellular matrix components, and remodeling enzymes of the extracellular matrix (28).

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**Inducers**
- Replicative senescence (RS)
  - Telomere attrition
- Oncogene-induced senescence (OIS)
  - Hras V12
  - NF-κB
- Oncogene-invalidation-induced senescence (OIS)
  - Myc
  - NF1
- Stress-induced premature senescence (SIPS)
  - Oxidative stress
  - Genotoxic agents
  - Cytokines

**Signaling pathways**
- DDR
- p53/p21
- p16/Rb
- COX2/PGE2/EPs

**Chromatin modifications**
- Remodeling (HP1)
- Histone modification (H3K9me3)
- SAHF
- PML

**Metabolic processing**
- Autophagy
- Glycolysis
- ROS production

**Secretory stimulation**
- Cytokines
- Chemokines
- MMPs

**Senescence Markers**
- SA-β-gal
- DNA damage foci
- Secretome (SASP)
- Enlarged cell size
- p16 expression
- Lack of cell proliferation

Fig. 2. Models of senescence. Four types of inducers lead to common cellular senescence signature via progressive molecular and cellular changes.
stable growth arrest (27, 81). However, all oncogenic activations are not able to induce senescence and can, on the contrary, have opposed effects. For example, the impairment of c-Myc was shown to induce senescence (130). This type of senescence was qualified as oncogene-induction-induced senescence (OIS). In addition, the loss of some tumor suppressor genes such as PTEN or NF1 was shown to induce senescence as well (29). SIPS, OIS, and OIIS are independent from telomere attrition (128).

It is well established that the irreversible cell-cycle arrest typical of senescence is mainly controlled by the p53/p21WAF1 and/or p16INK4a/Rb pathways. p53 is activated as a result of the persistent activation of the DNA damage response (DDR) pathway (61, 105). One cause of the activation of the DDR pathway is the erosion of telomeres. At a critical small size, the telomere is structurally disorganized (47), and the extremity of the chromosome is detected as a double-strand break, resulting in the activation of the DDR pathway (1). Since shortened telomeres are irreparable, the DDR signalization is persistent and the cell-cycle arrest becomes irreversible (39). The transcription factor p53 operates by inducing the expression of the cyclin/Cdk inhibitor p21WAF1, which blocks the cell cycle at the G1/S transition. The DDR signalization is also one major effector mechanism of OIS. It results from a replicative stress due to the hyperproliferation induced by the oncogenic activation (88). In addition to inducing the senescent cell-cycle arrest, DNA damage is also involved in the SASP. The signaling from the foci of DNA damage activates the NF-kB transcription factor, which in turn activates its target genes among which are the inflammatory cytokines composing part of the SASP (89, 107). Although the p16INK4a/Rb pathway seems to be a very universal inducer of the senescent cell-cycle arrest, the causes of the p16INK4a upregulation are still rather elusive. Like p21WAF1, p16INK4a is a cyclin/Cdk inhibitor, which blocks the cell cycle at the G1/S transition. Depending on the cellular context and/or the mechanism inducing senescence, the p53 and p16INK4a pathways act in parallel or are interdependent (5a, 61).

Another central mechanism in senescence is oxidative stress. It occurs in SIPS, OIS, and OIIS and can also act as a superimumerary mechanism in parallel to telomere shortening during replicative senescence (70, 80, 119). Oxidative stress induces nontelomeric DNA damages (119) and helps destabilizing the structure of telomeres (139). Although not definitely demonstrated, oxidative stress could be one activator of the p16INK4a/Rb pathway (12) through the upstream p38 MAPK (74, 132). In addition, oxidative stress also causes much damage to several cellular constituents including mitochondria and proteins (2, 72). It could hence be involved in the morphological and metabolic changes associated with senescence, but this remains to be formally demonstrated.

Several other pathways were shown to be involved in several senescent features including cell-cycle arrest, SA-β-Gal, or SASP. For instance, we and others reported that the cyclooxygenase 2 (COX-2)-prostaglandin pathway is involved in the induction and in the maintenance of the senescent phenotype of skin fibroblasts (32, 45, 138). This occurred through an independent-reactive oxygen species (ROS) and a dependent-PGE2/EP intracrine pathway (71). In addition, autophagy was demonstrated by the group of Narita to be an effector mechanism of OIS in fibroblasts. Inhibition of autophagy delayed the senescent phenotype, including the SASP (136). Our laboratory showed that autophagy is induced at a high level in senescent keratinocytes, as a consequence of their high level of oxidative damage to nuclei and mitochondria (35, 43). Similarly, human umbilical vein endothelial cells treated with glycated collagen I entered premature senescence associated with an increase of autophagosome formation as evidenced by overexpression of cleaved LC3 protein. Pharmacological inhibition of autophagy by 3-methyladenine prevented premature senescence (92). Moreover, a recent study proposed that metabolism reprogramming through, in part, the activation of autophagy allows cells to cope with the high demand of SASP factors in a chemotherapeutic-induced senescence model (38).

Senescence was shown to occur in vivo in different tissues, in different organisms and in different physio-pathological situations (61). Cells harboring senescent markers were shown to accumulate with age, notably in the skin (36, 37) but not in the heart, skeletal muscle, and kidneys (126). Baker et al. (3) demonstrated that this accumulation of senescent cells in tissues with aging is directly responsible for some age-related dysfunctions. Therefore, cellular senescence has been proposed as one of the nine hallmarks of aging (66). Paradoxically, senescent phenotypes were recently reported during embryonic developmental processes (116). Senescence also occurs in various pathologies. For example, some features of senescence have been described in glia and neurons in Alzheimer disease (20). Obesity was associated with fat tissue senescent cell accumulation (118), and inflammatory disease such as atherosclerosis shows elevated senescent markers in peripheral endothelial cells (125). Senescent cells were also found in benign tumors (26, 77), in the context in which oncogenes were altered, validating in vivo the concept of OIS. Importantly, senescent cells are present in benign tumors but lost in advanced tumors, supporting the idea that OIS is activated upon an oncogenic stress to suppress the tumor development (26, 77). Therefore, senescence is assumed to be a tumor suppressor (17) and this relies mainly on the persistent activation of the DDR pathway (102). However, the SASP that is enriched in inflammatory cytokines was shown in contrast to promote the development of precancerous cells (18).

**UPR Is a Component of the Senescent Phenotype**

Taking into consideration its main mechanisms, its main inducers, and its main physio-pathological occurrence, senescence can be viewed as a stress response phenotype that halts altered cells. This raises the question of whether the activation of another stress response pathway such as the UPR may occur during senescence, may explain some of the senescence features, or may even be an initial causal event.

The group of Soengas established in 2006 (34) that the activation of oncogenic HRasG12V in human melanocytes engaged a senescent phenotype associated with massive expansion and vacuolization of the ER. ER ultrastructure alterations were also reported in a model of adriamycin-induced senescence in lymphoma cells (38). Therefore, an ER stress would occur in correlation with senescence. In consequence, the UPR could be activated. Indeed, numerous studies reported such activation (Table 1). The analysis of the data from these studies does not highlight any differences regarding the cell type. For example, increased expression of several ER chaperones were
reported in cells as different as fibroblasts (11, 24, 75, 78), endothelial cells (57, 90), macrophages (5), melanocytes (34), keratinocytes (142), or renal epithelial cells (65). Similarly, the activation of the UPR seems to occur in all types of senescence, whether the inducer is successive replications (7, 11, 57, 75, 112), oncogene activation (34, 38, 142), DNA-damaging agents such as X-rays (90) or adriamycin (38), or oxidative stress (75). However, the precise signature of ER stress varies according to the context. For example, BiP/Grp78 mRNA and protein expressions were shown to be upregulated in several models of senescence but not in all (see Table 1). Another example is the protein disulfide isomerase PDIA1, involved in the formation of disulfide bonds, whose expression increases in senescent human umbilical vein endothelial cells (57, 75) but declines in senescent human dermal fibroblasts (11) and WI38 (75). Very few in vivo data are available. A study reported that spitz naevi-containing melanocytes expressing mutant HRasV12 displayed increased expression of BiP/Grp78 and PDI (34).

Regarding which arm of the UPR is activated during senescence, all three are activated (Table 2). However, the three branches are not always activated together in a given senescence context, and none of them seem specific for a type of senescence. A recent article highlighted that all arms of the UPR were activated and associated to replicative senescence in WI38 cells, but, in the same cells induced in senescence by H2O2, only the PERK branch was activated (75). The involvement of the PERK pathway in senescence is supported by numerous studies revealing the upregulation of CHOP/Gadd153, an ATF4 target gene (see Table 2). Moreover, ATF4

Table 1. ER stress genes and proteins whose expression is altered in senescence models

<table>
<thead>
<tr>
<th>Model of Senescence</th>
<th>Inducer</th>
<th>Cell/Animal Model</th>
<th>UPR Target Genes Modulated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicative</td>
<td>Human dermal fibroblasts</td>
<td>CNX protein expression decreased (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HUVECs</td>
<td>Calreticulin, PDIA1 protein expression increased, GRP94 protein expression decreased (57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human dermal fibroblasts</td>
<td>Calreticulin, BIP/GRP78, PDI protein expression decreased (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat embryo fibroblasts</td>
<td>α-Glucosidase II upregulated (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HUVECs and BJ fibroblasts</td>
<td>mRNA of Chop/Gadd153 found upregulated (112)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human fibroblasts</td>
<td>ER-resident chaperone HSP47 is decreased (78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primary kidney macrophage</td>
<td>Decreased Bip/Grp78 mRNA (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WI38 cells</td>
<td>Increased expression of BIP/GRP78 and ERO1Lo (75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased expression of CNX and PDI (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncogene-induced</td>
<td>H-Ras V12</td>
<td>Melanocytes</td>
<td>Increased Bip/Grp78, Chop, Atf4 protein expression (34)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>H-Ras V12</td>
<td>Increased of phosphorylated PERK, Increase of the sXPB1 mRNA form (142)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary murine keratinocytes</td>
<td>Increased CNX, ATF6 and Ph-Erk protein expression (142)</td>
<td></td>
</tr>
<tr>
<td>DNA damage-induced</td>
<td>X-rays</td>
<td>Pulmonary artery endothelial cells</td>
<td>Increased BIP/GRP78, CHOP, and GADD34 mRNA transient phosphorylation of eIF2α (90)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adriamycin</td>
<td>Lymphoma cells</td>
<td>Increased ATP4 and CHOP protein expression (38)</td>
<td></td>
</tr>
<tr>
<td>Drug/chemical/stress-induced</td>
<td>AGE, TG, SA-β</td>
<td>Proximal tubular epithelial cells</td>
<td>Increased BIP/GRP78 protein expression (65)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>MDA-MB-468</td>
<td>Increased mRNA expression of CHOP (129)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetrasubstituted Naphthalene Diimide Analogs</td>
<td>MIA, PaCa-2 pancreatic cells</td>
<td>Increased mRNA expression of CHOP (76)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low glucose treatment</td>
<td>Normal human skin fibroblasts</td>
<td>Increased expression of ORP150 (59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2O2</td>
<td>WI38 cells</td>
<td>Increased expression of ERO1Lo, Decreased expression of CNX and PDI (75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CuSO4</td>
<td>WI38 cells</td>
<td>Increased expression of BIP/GRP78 and ERO1Lo (75)</td>
<td></td>
</tr>
</tbody>
</table>

ER, endoplasmic reticulum; UPR, unfolded protein response; CNX, calnexin; TG, thapsigargin; SA-β, tunicamycin; AGE, advanced glycation end-product; RAGE: receptor of AGE; PERK, PKR-like ER kinase; HUVECs, human umbilical vein endothelial cells; MEFs, mouse embryonic fibroblasts.
Is It a Driver of Cell Senescence?

Is the UPR Activated in Consequence to Cell Senescence or Is It a Driver of Cell Senescence?

Therefore, it is clear that ER stress and activation of the UPR are components of the senescent phenotype. However, because of the diversity in the UPR signature and the versatility in the activation of the three UPR branches, the question arises of whether ER stress and the UPR are cause or consequence of cell senescence. Several studies where some components of the UPR were genetically of pharmacologically manipulated can help to answer this question. They are listed in Table 2, and the effects of the three UPR branches as inducer or repressor of senescence are recapitulated in Table 3.

Table 2. Alteration of the senescence features in ER stress-impaired models

<table>
<thead>
<tr>
<th>Branch</th>
<th>Model</th>
<th>ER Stress Modulation</th>
<th>Senescence Characteristic</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERK</td>
<td>HRas-driven senescence in melanocytes</td>
<td>ATF4 siRNA</td>
<td>Reduced the % of SA-β-Gal-positive cells</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>HRas-driven senescence in primary murine keratinocytes</td>
<td>ATF4 siRNA</td>
<td>Triggered senescence by expressing constitutively p16INK4 and p19ARF</td>
<td>(53)</td>
</tr>
<tr>
<td></td>
<td>HT1080 human fibrosarcoma</td>
<td>E235: activator of ATF4 expression at both mRNA and protein levels</td>
<td>Increase in perinuclear SA-β-Gal staining</td>
<td>(108)</td>
</tr>
<tr>
<td></td>
<td>B16F10 mouse melanoma</td>
<td>PERK siRNA</td>
<td>Increased the % of SA-β-Gal-positive cells.</td>
<td>(56)</td>
</tr>
<tr>
<td></td>
<td>Human endometrial stromal cells (ESCs)</td>
<td>Calreticulin siRNA</td>
<td>Increased the % of SA-β-Gal-positive cells.</td>
<td>(62)</td>
</tr>
<tr>
<td>IRE1α</td>
<td>HRas-driven senescence in melanocytes</td>
<td>XBP1 siRNA DN-IRE1α</td>
<td>Reduced the % of SA-β-Gal-positive cells</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td>H2O2-induced senescence in WI38 cells</td>
<td>Chemical inhibitor GSK2606414</td>
<td>Increase in p21 protein expression</td>
<td>(75)</td>
</tr>
<tr>
<td></td>
<td>HRas-driven senescence in primary murine keratinocytes</td>
<td>XBP1 siRNA</td>
<td>Reduced the % of SA-β-Gal-positive cells</td>
<td>(34)</td>
</tr>
<tr>
<td>ATF6α</td>
<td>HRas-driven senescence in melanocytes</td>
<td>DN-ATF6α</td>
<td>Reduced the % of SA-β-Gal-positive cells</td>
<td>(34)</td>
</tr>
<tr>
<td>All</td>
<td>HRas-driven senescence in primary murine keratinocytes</td>
<td>TG, SA-β</td>
<td>Reduced p16 and Dcr2 protein expression</td>
<td>(102)</td>
</tr>
<tr>
<td></td>
<td>RAGE-induced senescence</td>
<td>4-Phenybutylate (4-PBA) (UPR inhibitor)</td>
<td>Reduced the ratio of SA-β-Gal, SAHF-positive</td>
<td>(65)</td>
</tr>
</tbody>
</table>

Table 3. Role of ER and UPR components in senescence

<table>
<thead>
<tr>
<th>ER-Related Factor</th>
<th>Cellular or Senescence Model</th>
<th>Effect in Senescence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATF4</td>
<td>OIS</td>
<td>Both + and −</td>
<td>(33, 56, 145)</td>
</tr>
<tr>
<td>ATF6</td>
<td>OIS</td>
<td>+</td>
<td>(109)</td>
</tr>
<tr>
<td>Calreticulin</td>
<td>Normal cells</td>
<td>−</td>
<td>(33)</td>
</tr>
<tr>
<td>eIF2α</td>
<td>Normal cells</td>
<td>−</td>
<td>(102)</td>
</tr>
<tr>
<td>IRE1α</td>
<td>OIS</td>
<td>+</td>
<td>(102)</td>
</tr>
<tr>
<td></td>
<td>SIPS</td>
<td>+</td>
<td>(77)</td>
</tr>
<tr>
<td>PERK</td>
<td>Cancer cell lines</td>
<td>−</td>
<td>(58)</td>
</tr>
<tr>
<td></td>
<td>SIPS</td>
<td>−</td>
<td>(102)</td>
</tr>
<tr>
<td></td>
<td>SIPS</td>
<td>+</td>
<td>(77)</td>
</tr>
<tr>
<td>XBP1</td>
<td>OIS</td>
<td>Both + and −</td>
<td>(33, 145)</td>
</tr>
</tbody>
</table>

OIS, oncogene-induced senescence; SIPS, stress-induced premature senescence; +, inducer effect; −, repressor effect.
Arguments in favor of the UPR being a driver of senescence.

The three branches have shown positive relationships between their activation and cellular senescence (Tables 2 and 3). The chemical chaperone 4-PBA (an UPR inhibitor) reduced the number of SA-β-gal positive cells in receptor for advanced glycation end product-induced senescence of proximal tubular epithelial cells (65). Conversely, bortezomib, a known activator of the proapoptotic PERK arm of the UPR (86), was shown to transiently induce senescence before cell death in primary culture of NK lymphomas (113). Silencing ATF6α, ATF4, or XBP1 in melanocytes expressing HRasV12 drastically reduced the percentage of SA-β-Gal-positive cells and protected against the structural disorganization of the ER occurring during this type of senescence (34).

However, how ER stress and UPR could contribute to induce senescence? The level of oxidative stress is increased at senescence and is one of the mechanism inducing senescence (61). However, it is still unknown why oxidative stress increases in several situations of senescence. An overactivity of the ER could be a source for senescence-associated oxidative stress. Indeed, the folding of proteins in the ER, in particular the establishment of disulphide bonds, involves ER resident protein disulphide isomerases (PDI) and ER oxidoreductin 1 (ERO1) (122). ERO1 is a flavoenzyme that uses oxygen as an electron acceptor during the formation of disulfide bonds. This leads to the production of the reactive oxygen species H2O2 (50), which can diffuse all over the cell and could contribute to induce senescence. Moreover, the step of disulfide bond isomerization, which is necessary for properly positioning disulfide bonds, necessitates the consumption of a reduced glutathione (GSH) (50), hence, compromising the overall antioxidant defenses of the cell.

Autophagy might be another mechanism by which UPR could induce senescence. Indeed, on the one hand the induction of autophagy by sustained or unresolved UPR is well documented (8, 58, 87, 95, 103), and on the other hand it is demonstrated that autophagy actively contributes to senescence (35, 43, 137). However, presently no study demonstrates a direct cascade where the activation of the UPR would induce an autophagic activity, which itself would induce the onset or the maintenance of senescence.

It is robustly established that the p53/p21WAF1 pathway activated following accumulation of DNA damage is the main mechanism of the cell-cycle arrest associated with senescence (17). However, the UPR could also participate in the induction of the senescent cell-cycle arrest pathway. Indeed, a recent article shows that stress-induced senescence can be promoted through the activation of an ER stress-dependent p21 signaling (65).

Arguments in favor of the UPR being activated in consequence to senescence and to counteract senescence. The above cited studies suggest that the UPR could be one of the inducers of senescence. However, ER stress and the UPR could in contrast be activated as a consequence of senescence. One feature of the senescent phenotype potentially able to induce ER stress and the UPR is the SASP. During senescence, the activity of the ER and other organelles of the secretory pathway has to increase to ensure the production of inflammatory cytokines, components of the extracellular matrix and remodeling enzymes, which constitute the SASP and which are overexpressed and oversecreted at senescence. The capacity of the ER to properly produce and process these proteins of the SASP could be overwhelmed, leading to ER stress and UPR. This was demonstrated in a recent study with a model of therapy-induced senescence applied to lymphoma cells (38). Another feature of the senescent phenotype potentially able to induce ER stress and the UPR is oxidative stress. The proper folding and the proper formation of disulphide bonds in proteins necessitate a controlled oxidant state and GSH content, which could be perturbed by the senescence-associated oxidative stress. Moreover, chaperones and foldases may be the target of this oxidative stress, hence impairing the ER folding capacity. For instance, carbonylation (an oxidative damage) of calreticulin and ERp29 was reported in senescent WI-38 human embryonic fibroblasts (4).

In consequence of the ER stress induced by senescence, the UPR could be activated to restore the ER homeostasis and this could potentially impact the maintenance of the senescent phenotype. In support of this, an increase of the number of SA-β-Gal-positive cells and reduced p21WAF1 protein expression were observed when XBP1 was silenced by siRNA in a model of HRas-driven senescence in primary murine keratinocytes (142). The same study also reported decreased ratio of nuclear to cytoplasmic XBP-1 in vivo in areas of 7,12-dimethylbenzanthracene-induced benign skin tumors positive for senescent markers (142). Moreover, they also found a negative correlation between p16 and BiP/GRP78 expression in senescent regions of human colon adenomas (142). Horiguchi et al. (53) reported that transformation of primary embryo fibroblasts with HRas and SV40 large T antigen triggered senescence but only upon deletion of ATF4. Similarly, impairing eIF2α enhanced the doxorubicin premature senescence in both cultured HT1080 cells and xenograft tumor assays (99). However, how the UPR could counteract senescence? The only available data are on a limitation of the accumulation of ROS. This could operate through one of the targets of the PERK kinase, the NRF2 transcription factor that controls the expression of genes that maintain the redox homeostasis (31). Recently, a study described an altered expression of NRF2 and HO-1, one of its redox controlling genes, during HIV-1-induced premature senescence in rat tissues (33).

Fig. 3. Interrelationship between UPR and senescence-associated processes.
Conclusion/Future Directions

In conclusion, although many lines of evidence support a strong connection between senescence and UPR, experiments are further needed to better characterize the molecular and functional links between these two programs, especially in vivo models in which data are presently very few. The senescence and UPR programs seem to make an interconnected network in which oxidative stress would be a central element responsible for an auto-amplification loop (Fig. 3). It should be particularly informative to better characterize the UPR contribution to the SASP. As already mentioned, the ER expansion occurring at senescence could be a consequence of the increasing demand of protein synthesis, maturation, and secretion necessary for the production of the SASP. One could assume in addition that, if the SASP is produced by a dysfunctional secretory pathway, it might contain abnormally folded and matured proteins which could perturb the organization and functioning of the microenvironment. This might be especially relevant regarding the detrimental impact of the SASP in aging and tumor development (17, 23, 60, 68). Another point that would deserve further investigations is the relationships among UPR, autophagy, and senescence. It is presently established that the autophagy occurring at senescence is the consequence of the accumulation of oxidatively damaged cell components (35). However, it is not excluded that the UPR could also be an inducer of autophagy at senescence. Moreover, the expanded ER could be an increased source of membranes necessary for the high production of autophagic vesicles. Conversely, autophagy could be activated to degrade by a specific ER-phagy mechanism the supernumerary ER membranes.

Data on the duration and strength of the ER stress occurring at senescence are also today lacking. Nevertheless, they might be critical determinants of whether UPR induce or reduce the senescent phenotype. It is likely that a severe ER stress may contribute to the onset of senescence and a mild/sustained one may help to the maintenance of the senescent phenotype. Alternatively, a mild/sustained ER stress may progressively induce senescence and then ensure its maintenance. It is also conceivable that a persistent/unresolved ER stress could lead to the death of senescent cells. As for a mild ER stress, it may help the senescent cell to restore its homeostasis and hence reduce its senescent features.

Therefore, although sounding like a promising avenue, all these points should be clarified before considering ER stress and UPR components as therapeutics targets to decrease the accumulation of senescent cells with aging and hence decrease the incidence of age-associated pathologies.

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