Unfolded Protein Response (UPR) Controls Major Senescence Hallmarks

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UNFOLDED PROTEIN RESPONSE (UPR) CONTROLS MAJOR SENESCENCE HALLMARKS

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Running title: UPR and senescence

Abstract

Senescence is a complex cellular state which can be considered as a stress response phenotype. However, the mechanisms through which cells acquire and maintain this phenotype are not fully understood. Here, we argue that the Unfolded Protein Response (UPR) may represent a signalling platform that is associated with the major senescence hallmarks.

Keywords (up to 6) :

Senescence, Endoplasmic reticulum, Unfolded Protein Response, secretome, metabolism, proteostasis

Senescence is a cellular state characterized by a stable proliferation arrest and accompanied by molecular changes, including epigenetic changes, changes in membrane lipid composition, increased oxidative stress, persistent DNA damage, increase in autophagic activity, and metabolic reprograming, as well as substantial morphological changes, such as cell enlargement. Remarkably, senescent cells secrete a wide array of proteins constituting the so-called senescence-associated secretory phenotype (SASP) which is enriched in pro-inflammatory cytokines, growth factors, and enzymes remodelling the extracellular matrix. Senescence can be induced by various types of stress, including telomere shortening, oxidative stress, exposure to radio- or chemotherapy, UV radiation, and activation of oncogenes [1]. Therefore, senescence can be regarded as an adaptive response to stress. Upon stress, cells trigger dynamic signalling pathways, including the unfolded protein response (UPR) elicited by the endoplasmic reticulum (ER). We and others have shown that ER stress/UPR activation occur at senescence [2]. Here, we discuss how these processes contribute to the senescent cell specific properties.

Evidence of altered ER homeostasis during senescence

The ER plays a key role in proteostasis and controls much of the membrane proteome and secretome. When ER homeostasis is disturbed by intrinsic and/or extrinsic stress, the UPR is induced as an adaptive response. It is initiated by the activation of three sensors (PERK, ATF6α, and IRE1α), which results in the induction of their effectors in order to counteract the proteostasis imbalance (**Box 1**). Different cell types undergoing senescence upon different kinds of stress display structural alterations of the ER and elicit the UPR activation. For example, dermal fibroblasts undergoing replicative senescence (RS) and melanocytes undergoing oncogene (HRAS)-induced senescence (OIS) present enlarged ER and the activation of the three arms of the UPR [3][4]. Lymphoma cells undergoing therapy-induced senescence (TIS) have disorganized rough ER cisternae and the activation of at least the IRE1α/XBP1 and PERK/ATF4 branches of the UPR [5]. However, other reports indicate that the UPR arms can be involved in senescence in a more differential manner. For example, in HRAS-driven senescent keratinocytes, IRE1α, but not PERK, was activated, and the IRE1α capacity to induce senescence implicated its RNAse activity independently of XBP1 splicing but through cleavage of *ID1* messenger RNA (mRNA) [6]. These few studies highlight that the UPR could be a major basic mechanism on which the senescent phenotype relies, however with

some subtleties and specificities in the involvement of the numerous complex UPR subpathways.

Evidence that UPR controls several senescence hallmarks

Since the UPR is activated at senescence, the questions are whether and how it contributes to the establishment or maintenance of the specific properties/markers of senescent cells. First of all, we and others reported that ER stress/UPR inducers (e.g. thapsigargin, dithiothreitol and UV) applied to proliferating cells induce all major senescent hallmarks (e.g. SA-β-Gal activity and DNA damage) [3][7]. However, converse approaches of invalidating the main UPR sensors or effectors in already senescent cells have given a more nuanced picture. For example, silencing ATF6α, IRE1α, or PERK in replicative senescent fibroblasts failed to alleviate the senescent cell cycle arrest [3]. In contrast, silencing PERK and ATF6α, but not IRE1α, in senescent keratinocytes, whose senescence is oxidative stress-dependent, significantly restored their proliferation [8]. In HRAS-induced senescence, the control of the cell cycle arrest by the UPR pathway is time-dependent. Indeed, HRAS activation induces hyperproliferation in the first one or two days, followed by senescence within five to ten following days. Knocking-down IRE1α reduced proliferation of the first phase, and blocked the induction of senescence and sustained proliferation during the second phase. This activity was shown to be independent of *Xbp1* splicing since XBP1 knock-down blocked proliferation and accelerated senescence [6].

The senescent cell cycle arrest is mainly the result of the persistence of unrepaired DNA damage. Interconnections between DNA damage/repair and ER stress/UPR in the context of senescence were, up to now, poorly investigated. However, based on transcriptomic profiling, a recent study predicted that ATF4, one component of the PERK pathway, might play a role in DNA damage repair during senescence [9]. Another obvious *in vitro* senescence marker is the increase in cell size and spreading. We have shown that the silencing of ATF6α, and ATF6α only, in replicative senescent fibroblasts reduced cell spreading and restored a fusiform shape similar to that of exponentially growing fibroblasts. Interestingly, these effects were correlated with reversed ER expansion [3].

The third widely recognized marker of senescence is the senescence-associated β-Galactosidase (SA-β-Gal) activity, reflecting the increase in lysosome and autophagic vacuole content. According to the cell type and senescence inducer, invalidation experiments highlighted either ATF6α and IRE1α, or ATF6α and PERK as the main factors responsible for increased SA-β-Gal activity [3][4][7][8]. Accordingly, silencing of ATF4 (PERK effector) in

TIS glioblastoma cells significantly decreased the level of MAP1LC3-II, which is indispensable to the autophagic activity [10].

Finally, regarding the SASP, a study indicated that ER stress may trigger a negative feedback loop attenuating the SASP gene expression [11], suggesting that ER stress/UPR activation might have an impact on the secretome composition. It was also shown that the increased demand for protein synthesis to ensure the SASP component production during HRAS-mediated senescence induces a proteotoxic stress and UPR activation [5][11].

These above-mentioned results raise the question of whether UPR is the cause or the consequence of senescence. The answer is probably that both are true because of additional layers of auto-amplification loops. Therefore, determining the temporal sequence of the cause-effect relationship between UPR and senescence will be the next challenge in the field. Another pending question is whether the proteotoxic stress (due to oxidized misfolded protein or SASP protein overload) is the only mechanism that induces/maintains UPR at senescence. Interestingly, changes in membrane lipid composition occur during senescence [12] and structural changes of the ER lipids can directly activate the ATF6α and IRE1α/XBP1s UPR arms. These two pathways, in turn, are known to activate lipid biosynthesis [13], indicating an interdependency between lipids and UPR, both of which may lead to cellular senescence independently of proteotoxic stress. Overall, these data support the hypothesis that UPR is a signalling platform associated with senescence hallmarks (**Figure 1**).

Modulating UPR to limit senescence and age-related diseases

Senescent cells accumulate during aging in most tissues and organs. Eliminating these senescent cells using a dedicated mouse model or senolytic drugs (drugs able to kill senescent cells) increased life span and decreased the incidence of most age-related pathologies [14]. The UPR is established as a survival mechanism enabling cells to resist and resolve stress, but also, when sustained because of a stress that remains unresolved, as a mechanism leading to cell death by apoptosis, mainly through the activation of the CHOP transcription factor [15]. In senescent cells, the UPR is activated in a long term, and CHOP is often activated [3][4][5][8][10][11], but, surprisingly, does not lead to senescent cell death. Therefore, senescent cells might require an optimal UPR activity (specific subpathways at specific levels of activation with specific kinetics) for their long-term survival. If this is true, pharmacological compounds inhibiting the UPR subpathways might act as senolytics. However, none of the above cited studies where some UPR pathways were invalidated led to this conclusion. In contrast, they have shown that invalidating the UPR led to a regression of the senescent

hallmarks. Therefore, inhibiting some of the UPR sensors or effectors (e.g. ATF6α in replicative senescence or IRE1α in Hras-driven senescence) by specific pharmacological compounds could be beneficial to delay, limit, or even suppress the deleterious aspects of senescence, in particular the inflammatory SASP. Hence, they would work as senomorphics, i.e. drugs able to suppress all or part of deleterious senescence hallmarks, and which could have less detrimental secondary effects than senolytics. Of course, further *in vivo* experiments are needed to better understand this therapeutic potential.

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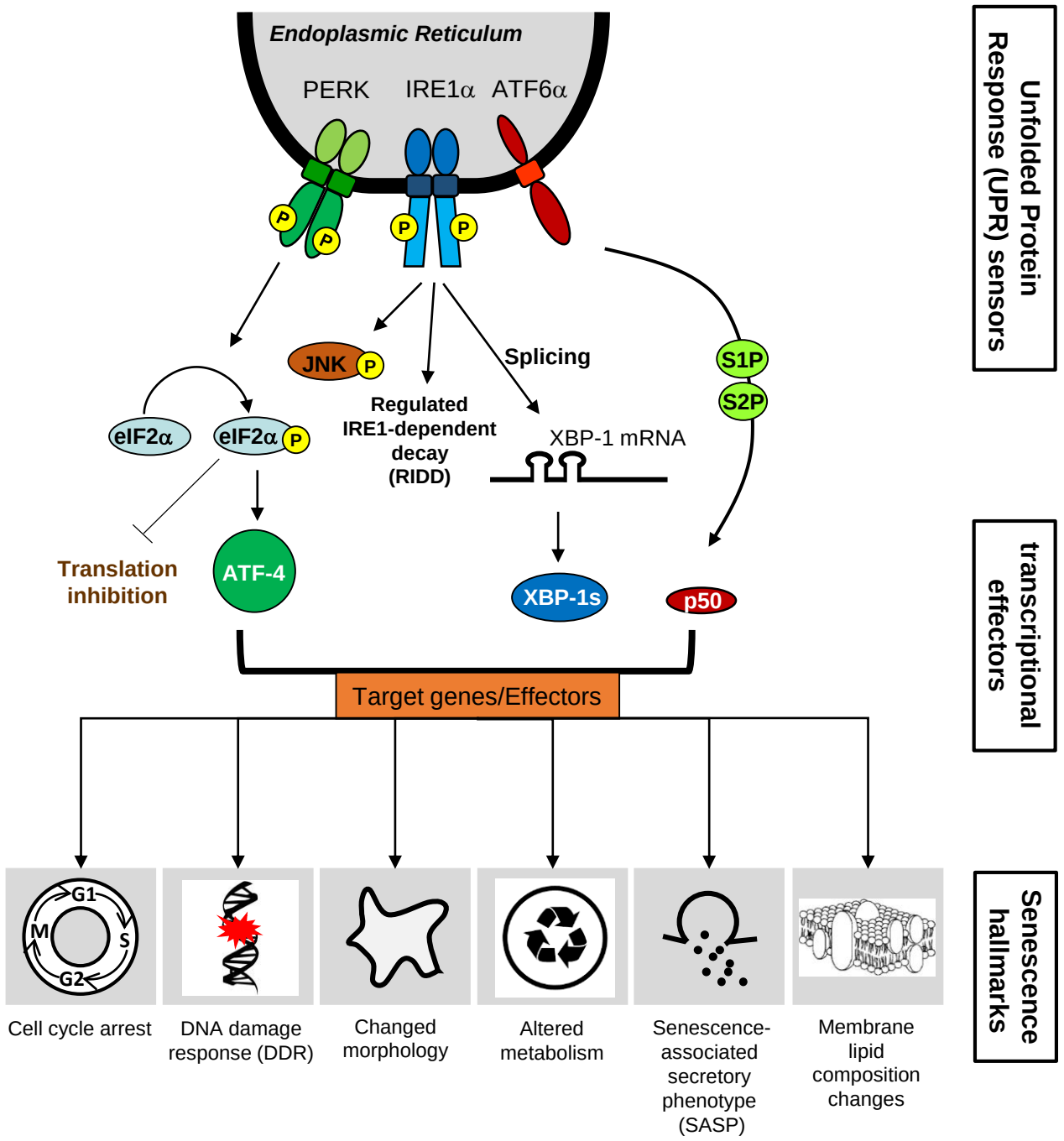
Box 1: Activation of the three arms of the UPR pathway

The UPR acts through three sensors: PERK, IRE1αand ATF6α. The PERK kinase reduces cap-dependent protein synthesis through eIF2α phosphorylation, and induces the transcription of genes encoding specific chaperones by activating the ATF4 transcription factor. Activated IRE1α induces unconventional splicing of the XBP1s mRNA which gives rise to an active transcription factor whose main targets are the genes whose products are involved in the quality control of the ER proteins. Other mRNAs are cleaved by IRE1α in a process called Regulated IRE1α-dependent decay (RIDD). However, the physiological consequences of this mechanism are not yet fully known. ATF6α, upon activation, exits the ER and integrates the Golgi apparatus membrane where it is cleaved by the Site-1 protease (S1P) and Site-2 protease (S2P) to its active form which acts as a transcription factor targeting genes encoding chaperones (Figure 1). If the cell adaptation through UPR is not possible due to prolonged or unresolved ER stress, cell death can be induced through the IRE1α/JNK or the ATF4 pathways.

Figure 1: Interconnection between UPR and senescence

All three ER stress sensors (PERK, IRE1α, and ATF6α) activate signaling events that lead to the attenuation of protein synthesis as well as to the induction of a specific transcriptional program both aiming to restore ER proteostasis and more broadly cellular homeostasis. Some of the UPR downstream signaling events, yet unknown, control the establishment and/or maintenance of the main senescence hallmarks including cell cycle arrest, DNA repair capacity, morphological changes, metabolic changes, the secretory pathway, and changes in membrane lipid composition. Arrows, positive regulation; Dead end arrow, negative regulation; JNK, c-Jun N-terminal kinase ; S1P/S2P, Site-1/2 protease; P, phosphorylation; ATF4, Activating Transcription Factor 4; XBP-1s, spliced form of X-box binding protein 1.


Endoplasmic Reticulum
PERK
IRE1α
ATF6α
P
JNK
Splicing
S1P
S2P
eIF2α
eIF2α
Regulated IRE1-dependent decay (RIDD)
XBP-1 mRNA
Translation inhibition
ATF-4
XBP-1s
p50
Target genes/Effectors
G1
S
G2
M
Cell cycle arrest
DNA damage response (DDR)
Changed morphology
Altered metabolism
Senescence-associated secretory phenotype (SASP)
Membrane lipid composition changes
Unfolded Protein Response (UPR) sensors
transcriptional effectors
Senescence hallmarks