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Review

The unfolded protein response as regulator of cancer stemness and differentiation: Mechanisms and implications for cancer therapy

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ABSTRACT

The unfolded protein response (UPR) is an adaptive mechanism that regulates protein and cellular homeostasis. Three endoplasmic reticulum (ER) membrane localized stress sensors, IRE1, PERK and ATF6, coordinate the UPR in order to maintain ER proteostasis and cell survival, or induce cell death when homeostasis cannot be restored. However, recent studies have identified alternative functions for the UPR in developmental biology processes and cell fate decisions under both normal and cancerous conditions. In cancer, increasing evidence points towards the involvement of the three UPR sensors in oncogenic reprogramming and the regulation of tumor cells endowed with stem cell properties, named cancer stem cells (CSCs), that are considered to be the most malignant cells in tumors. Here we review the reported roles and underlying molecular mechanisms of the three UPR sensors in regulating stemness and differentiation, particularly in solid tumor cells, processes that have a major impact on tumor aggressiveness. Mainly PERK and IRE1 branches of the UPR were found to regulate CSCs and tumor development and examples are provided for breast cancer, colon cancer and aggressive brain tumors, glioblastoma. Although the underlying mechanisms and interactions between the different UPR branches in regulating stemness in cancer need to be further elucidated, we propose that PERK and IRE1 targeted therapy could inhibit self-renewal of CSCs or induce differentiation that is predicted to have therapeutic benefit. For this, more specific UPR modulators need to be developed with favorable pharmacological properties that together with patient stratification will allow optimal evaluation in clinical studies.

1. Introduction

Proteins are the building blocks of organisms and key regulators of virtually all biological processes. Maintenance of protein homeostasis (proteostasis) is essential for proper cell function, development, growth and for organism health. Proteostasis control mechanisms, such as the unfolded protein response (UPR), ubiquitin–proteasome system (UPS) and autophagy, have been installed in eukaryotic cells during evolution to enable proper organism function and avoid pathological or lethal consequences [1–3].

Embryonic stem cells (ESCs) and adult stem cells (ADSCs) are at the basis of organism development and tissue homeostasis in the adult, respectively. ESCs have pluripotent differentiation capacity giving rise to a complete organism, whereas ADSCs are more restricted in differentiation capacity able to generate specific tissues or replenish damaged tissue cells [4]. Stem-like cells have been identified in cancers, commonly named cancer stem cells (CSCs), based on overlapping cellular properties such as self-renewal and differentiation potential [5]. CSCs are considered to be the most malignant tumor cells within a tumor, exhibiting resistance to commonly used therapies and deemed responsible for tumor relapse and metastatic disease. As such identification of molecular mechanisms that regulate CSCs and their highly adaptive abilities, is key for the development of novel treatments that, by eliminating CSCs, will effectively eradicate tumors and thus improve prognosis of cancer patients [6–8].

In this review we provide an overview of the involvement of the UPR in regulating stemness and differentiation in stem cells in general, but specifically in CSCs. Currently known molecular mechanisms of UPR-dependent stem cell regulation will be described and implications for cancer therapy will be discussed.

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2. The unfolded protein response

The endoplasmic reticulum (ER) is not only localized around the nucleus but extents to every other cellular organelle and the cell membrane. It is the central organelle for protein synthesis that includes productive protein folding and protein modifications, but also for regulation of protein trafficking and secretion, and is the main site for intracellular calcium storage and for the control of lipid homeostasis [9]. Moreover, the ER has been described to interact functionally and/or physically with many (almost all) other cellular compartments [10].

To ensure correct secretory and transmembrane protein production in the ER and thus cellular homeostasis, the UPR plays an essential role. The unbalance between the demand and capacity of ER protein folding leads to the accumulation of improperly folded proteins in this compartment, a situation known as ER stress that triggers the UPR as an adaptive response to restore ER proteostasis [11,12]. The accumulation of misfolded proteins beyond a tolerable threshold is monitored by three ER-resident transmembrane sensors, IRE1 (inositol requiring enzyme 1), PERK (double-stranded RNA-activated protein kinase (PKR)–like ER kinase) and ATF6 (activating transcription factor 6) [13–16] (see also Fig. 1). The activation of these sensors and signal transducers is generally attributed to their dissociation from the chaperone BiP (binding-immunoglobulin protein), also known as 78-kDa glucose-regulated protein (GRP78), since BiP is recruited to misfolded proteins to assist in restoring correct protein folding. However, the precise underlying mechanisms of their activation remain to be fully characterized.

ATF6 is activated upon ER stress and exported to the Golgi apparatus where it undergoes S1P/S2P protease cleavage yielding transcriptionally active ATF6f that triggers the expression of chaperone-coding genes and genes whose products are involved in ER-associated protein degradation (ERAD) [17].

PERK, a serine/threonine protein kinase, undergoes oligomerization and auto-phosphorylation upon ER stress and subsequently phosphorylates the eukaryotic translation initiation factor 2a (eIF2α) resulting in attenuation of mRNA translation thereby preventing further entry of newly synthesized proteins in the stressed ER. This translation attenuation also favors the translation of mRNAs with short open reading frames in their 5′-untranslated regions, such as ATF4. The latter is a transcription factor that triggers the expression of genes whose products enhance protein folding capacity, amino acid metabolism, autophagy or antioxidant response. Among the ATF4 targets is C/EBP-homologous protein (CHOP) and together they induce the expression of Growth arrest and DNA damage-inducible gene 153 (GADD34), a phosphatase subunit that together with Protein Phosphatase 1c (PP1c) can reverse eIF2α phosphorylation and restore mRNA translation [18]. In addition, CHOP expression increases further when proteostasis cannot be restored leading to cells switching into a cell death modes [19]. At last, NF-E2-related factor-2 (NRF2), a Basic Leucine Zipper Domain (bZiP) protein, has also been described as a PERK kinase substrate and its phosphorylation-dependent activation regulates the cellular antioxidant response which is required to balance increases in reactive oxygen species (ROS) levels linked with ER stress [20].

IRE1 exhibits both kinase and endoribonuclease activities in its cytosolic region that are activated after oligomerization. IRE1 ribonuclease (RNase) activation leads, together with the tRNA ligase RTCB [21], to the non-conventional splicing of the mRNA encoding X-box binding protein 1 (XBP1) yielding XBP1s, a transcription factor that regulates the expression of ERAD components, chaperones, foldases and

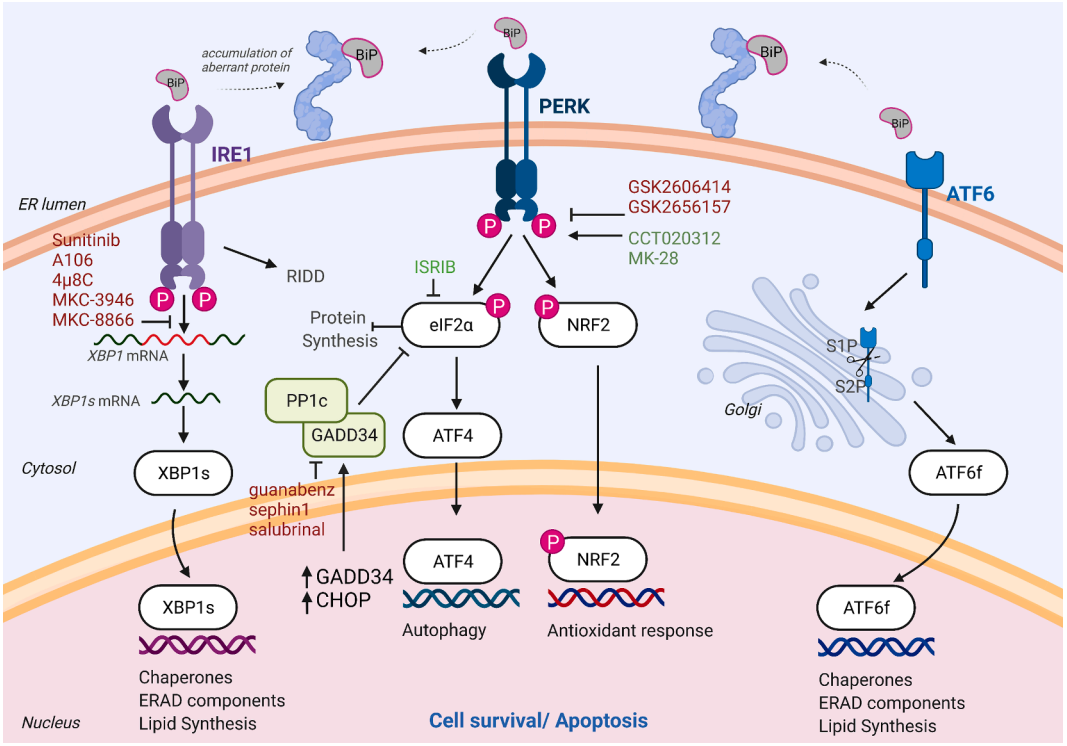

Fig. 1. The three branches of the Unfolded Protein Response Simplified representation of the core of the classic UPR. Cells under ER stress activate the adaptive UPR to maintain proteostasis and cellular homeostasis. Three sensors are activated in the ER lumen after dissociation from the chaperone BiP that is recruited to accumulating misfolded proteins. IRE1 oligomerization and auto-phosphorylation induces kinase and RNase activity leading to mRNA degradation (RIDD) and altered splicing of the XBP1 mRNA and production of the transcription factor XBP1s. PERK undergoes oligomerization and auto-phosphorylation upon BiP release and subsequently phosphorylates the eukaryotic translation initiation factor eIF2α resulting in transient attenuation of global protein translation and translation of specific sets of mRNAs such as of transcription factor ATF4. NRF2 is also phosphorylated by PERK and activates anti-oxidant responses. ATF6 activation occurs after transport to the Golgi apparatus where it undergoes S1P/S2P protease cleavage yielding transcriptionally active ATF6f. For IRE-1 and PERK pharmacological modulators, inhibitors (green/ gray) and activators (red/ black), are indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lipid biosynthesis enzymes. IRE1 RNase activity is also involved in the cleavage and/or degradation of RNAs, known as regulated IRE1-dependent decay (RIDD), including mRNA, rRNA or non-coding RNA [22]. The three UPR branches have separate and overlapping functions and together orchestrate cellular proteostasis and cellular homeostasis.

3. The UPR in cancer

The UPR has been found to be involved in various pathologies including metabolic and inflammatory diseases, degenerative disorders and cancer [23]. In cancer, the UPR has been implicated in various aspects including carcinogenesis, cancer malignancy and chemo/radiotherapy resistance, often having a cell survival function but also depending on ER stress levels as a mediator of cell death [24–26]. Cancer cells are exposed both to intrinsic and extrinsic ER stress conditions. For example, oncogenic transformation is accompanied by a high demand for protein synthesis and aberrant protein production (*intrinsic stress*). Restrictive conditions in the tumor microenvironment (TME) such as nutrient shortage, hypoxia or exposure to chemotherapeutics (*extrinsic stress*) all generate ER stress and require the UPR for maintaining proteostasis and thus tumor cell viability. To control cellular homeostasis and cell survival the UPR is generally highly active in cancer cells [27]. The UPR has been found to contribute to various hallmarks of cancer that include genome instability, sustained proliferation, cell death resistance, angiogenesis induction, immune suppression, inflammation, invasion and metastasis [27,28]. These hallmarks cannot all be directly linked to the proteostasis maintaining function of the UPR. Recent findings have indicated that the UPR has also other important functions whereby physiological stimuli are processed by the UPR and regulate a diversity of processes such as metabolism, immunity and cell fate [29–32]. A number of co-regulators of the UPR have been identified that are able to modify or interact with the ER sensors forming a complex designated the UPRosome, in which IRE1 and PERK function as scaffolds that mediate signal transduction [30,31]. The more precise nature and cell type-dependency of the physiological stimuli that activate the UPR and the molecular mechanisms responsible for the different cellular outputs remain to be identified.

4. The UPR in development and stem cells biology

The UPR genes were discovered to be crucial for the development of various metazoans from *Drosophila* to humans [33]. Moreover, transient activation of the UPR was reported to be essential for Yamanaka transcription factor (Octamer-binding transcription factor 4 (OCT4), Sex determining region Y-box 2 (SOX2), Kruppel-like factor 4 (KLF4), and Myelocytomatosis oncogene (c-MYC)) dependent reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) [34]. All three branches appear to be involved in developmental processes and non-redundant roles were suggested by the observation that impairment of individual branches can have different effects on tissue development, for example in pancreas development in mice [35,36]. Studies have shown that ER stress and the UPR regulate differentiation of various tissues, including in erythropoietic, chondrogenic, osteogenic, intestinal and neural differentiation as reviewed elsewhere [33,37–40]. Since stem cells are key players in developmental processes it was perhaps not surprising that the UPR was found to regulate cellular stemness and differentiation. For example, the UPR has been reported to contribute to the survival of murine hematopoietic stem cells (HSCs) since conditional knockout of *Grp78* resulted in HSC depletion accompanied by activation of all three UPR branches and apoptosis [41]. Similarly, in human HSC as well as in leukemic SC the IRE1-XBP1 pathway was found to reduce ER stress, increase cell survival and promote carcinogenesis [42]. In murine intestinal epithelium, low ER stress/UPR activity was observed in intestinal stem cells (ISCs) whereas differentiation was accompanied by UPR activation in which PERK-eIF2α was instrumental [43]. *In vitro* ISCs could be differentiated by exposure to ER stress inducers and *in vivo* conditional loss of *Grp78* in murine ISCs also induced differentiation. Similarly neural differentiation of bone marrow stromal cells and mouse ES cells was accompanied by UPR activation, and ER stress could trigger neuronal differentiation *in vitro* [44]. Together, these findings illustrate that ER stress and the UPR are important regulators of tissue development, stem cell maintenance and differentiation.

5. The UPR in cancer stem cells

Cancer stem cells (CSCs) have been identified in many tumor types and are considered to be the most malignant cells within a tumor [45,46]. Representing undifferentiated cells and endowed with self-renewal potential, CSCs are thought to form the root of the tumor and are deemed essential for colonizing tissues during the metastatic process. They are highly clonogenic and tumorigenic as demonstrated by *in vitro* and *in vivo* experiments, where they are able to reestablish a tumor with properties similar to that of the original tumor after reimplantation, giving rise to heterogeneously differentiated tumor cells. These features, together with their chemo- and radiotherapy resistance phenotype, have made CSCs key targets for the development of new and more effective treatments [6]. Various molecular pathways have been identified that regulate stemness and differentiation of CSCs, often overlapping with pathways involved in non-cancerous stem cell maintenance. Considering the importance of the UPR in regulating these processes, this adaptive pathway has also been studied for affecting the differentiation status of tumor cells and the regulation of CSCs. This is further illustrated for a number of solid tumor types below (see also Fig. 2).

5.1. UPR, tumor formation and stemness in breast cancer

Increased UPR activity has been reported in metastatic breast cancer cells in the bone marrow of patients, likely facilitating colonization. These cells showed overexpression of UPR proteins BiP, GRP94 and protein-disulfide isomerase (PDI) and, moreover, also were characterized by a high proportion of CD44(high)/CD24(low) cells known to mark breast CSCs [47]. *In vitro*, exposure of these metastatic breast cancer cells to either hypoxia or hypoglycemia, resembling harsh conditions of the TME, resulted in upregulation of BiP and a more mesenchymal cellular phenotype that allowed cells to cope better with the experienced stress conditions [48].

Another study showed an important role for PERK in mammary tumor formation, a key property of CSCs. Knockdown of PERK in mammary carcinoma cells isolated from Mouse mammary tumor virus (MMTV)-Neu transgenic mice demonstrated strongly reduced tumor growth compared to control cells upon mammary fat pad implantation [49]. Underlying mechanisms were examined and revealed a G2/M delay and reduced growth rates of PERK depleted human breast cancer cells as well as increased ROS levels. Elevated ROS levels caused DNA damage likely responsible for cell cycle arrest as part of the DNA repair response. The PERK substrate and redox state regulating kinase Nrf2 appeared to be instrumental since stable transfection of Nrf2 restored normal proliferation in PERK-depleted breast cancer cells.

PERK and ATF6 expression were also reported to positively correlate with both grading and staging of breast cancers as determined by immunohistochemical analyses of breast cancer tissue microarrays, similar as found for the stem cell transcription factor SOX2 [50]. IRE1 correlated only with progressive grading and BiP with staging of breast cancers. Experiments in mammospheres derived from breast cancer cell lines revealed that the UPR sensors expression correlated with stem cell markers expression and that pharmacological inhibition of all three UPR sensors as well as ER stress could reduce proliferation that was exacerbated under nutrient starvation. Particularly PERK and ATF6 inhibition reduced mammospheres formation of MDA-MB-231 cells and mechanistic studies revealed that silencing of PERK and ATF6, but not IRE1, strongly reduced SOX2 levels suggesting their importance in breast CSC maintenance. OCT4 and SOX2 proteins were found to associate with

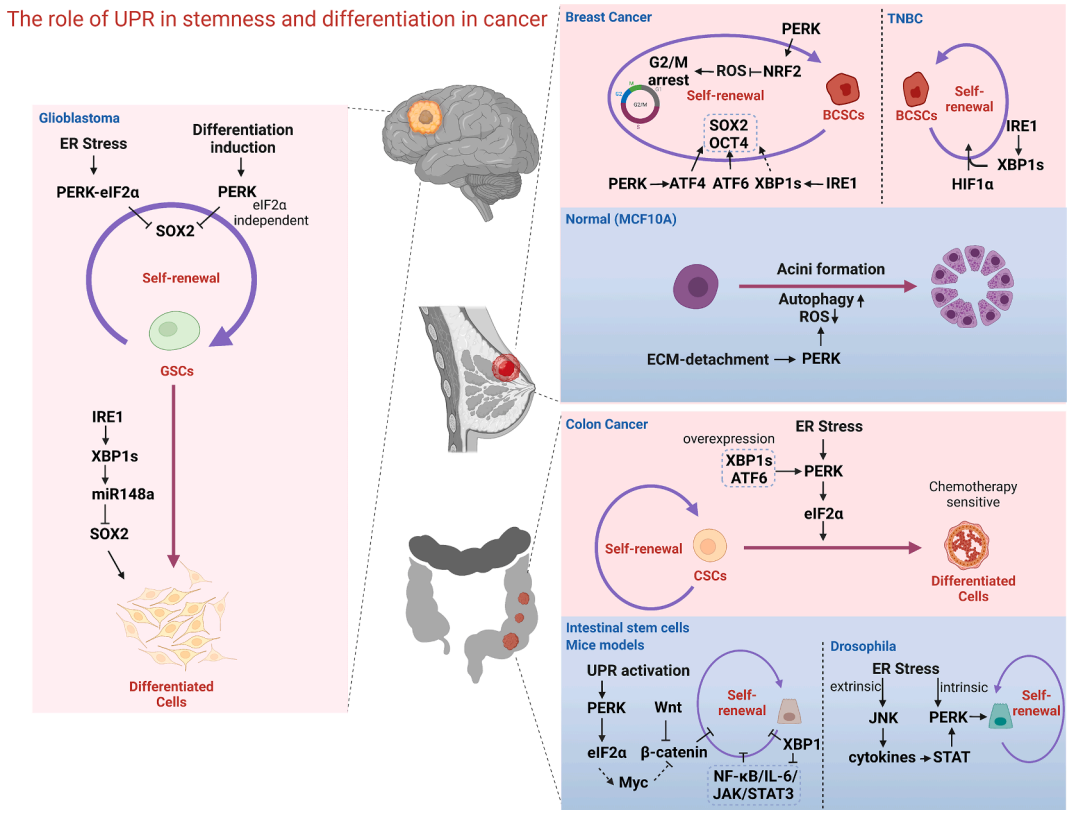

Fig. 2. The UPR regulates self-renewal and differentiation in cancer Summarizing overview of the reported UPR sensor-mediated regulation of self-renewal/ proliferation and differentiation in CSCs and during tumorigenesis. Examples of currently known mechanisms are shown for breast cancer, colorectal cancer and glioblastoma. In glioblastoma applied ER stress (tunicamycin) reduced self-renewal of GSCs, mediated by PERK/ eIF2α resulting in SOX2 downregulation. In addition, PERK was required for normal serum-induced differentiation of GSCs also involving SOX2 downregulation, but independent of eIF2α via a yet unknown mechanism. On the other hand, IRE1 activity, particularly of XBP1s, maintained a differentiated phenotype via XBP1s-dependent upregulation of miR148a reducing SOX2 levels. In breast cancer, PERK-ATF4, IRE1-XBP1s and ATF6 stimulated BCSCs by enhancing SOX2 and OCT4 expression. Furthermore, NRF2, a direct substrate of PERK, can alleviate ROS production and subsequent G2/M arrest, thus facilitating BCSC activity. IRE1/ XBP1s enhanced tumorigenicity and progression of TNBC. XBP1s in cooperation with HIF1α stimulated stemness of TNBC cells. On the other hand, acini formation by normal MCF10A cells was dependent on ECM-detachment induced PERK activation and subsequent autophagy activation, ROS reduction and anoikis suppression. Impairment of this process is thought to contribute to tumorigenesis. In normal intestine and colon cancer the UPR also regulates stemness. ER stress induction (subtilase cytotoxin AB) in colon-CSCs resulted in UPR activation and decreased stemness associated with activation of all three branches with PERK-eIF2α being instrumental. In Drosophila, cell intrinsic ER stress enhanced ISC activity via PERK activation, whereas ER stress in neighboring cells (extrinsic) indirectly stimulated ISCs by activating JNK-dependent secretion of cytokines leading to STAT and subsequent PERK activation in ISCs. In mice, XBP1 deficiency resulted in enhanced proliferation of ISCs. XBP1 suppressed self-renewal of ISCs by inhibiting an NF-kB/IL-6/JAK/STAT3 activation loop. In murine ISCs the Wnt/ß-catenin maintains stemness. PERK-eIF2α mediates ISC differentiation and counteracted ß-catenin activity in the nucleus likely via PERK-eIF2α dependent downregulation of c-Myc expression. Overall, the role of the individual UPR branches can differ between established CSCs and during tumor formation, either tumor promoting or suppressing, also depending on tumor specific properties.

ATF4 and ATF6, but less to XBP1s, in co-immunoprecipitation experiments but the more precise mechanisms by which PERK and ATF6 regulate stemness remains unclear. Another study reported the importance of XBP1s in tumorigenicity and progression of triple-negative breast cancer (TNBC) *in vitro* and found that XBP1s stimulated stemness of TNBC cells, involving cooperation at the transcriptional activation level between XBP1s and Hypoxia-inducing factor 1α (HIF1α) [51].

Apparently in contrast with elevated UPR sensor activity in breast CSCs are studies demonstrating that PERK signaling is required for proper mammary epithelial development into acini and prevents malignant transformation into breast cancer [52]. Loss of substrate adhesion of normal breast MCF10A cells, mimicking the process of mammary acini development, induced PERK activation and subsequent eIF2α-dependent upregulation of ATF4 and GADD153/CHOP. PERK inhibition resulted in cellular hyperproliferation and altered acini development *in vitro* and overexpression of dominant negative kinase dead PERK in MCF10A stimulated tumor formation. A follow up study of the same group showed that extracellular matrix (ECM)-detachment induced PERK activation is instrumental for autophagy activation and ROS reduction thus suppressing anoikis and facilitating proper acini formation [53]. In addition, ECM-detachment was found to induce autophagy by PERK mediated activation of LKB – AMPK (AMP-activated protein kinase)-dependent inhibition of mammalian target of rapamycin complex 1 (mTORC1) [54].

The findings above may indicate that the UPR could have different functions in the onset of tumorigenesis and in established breast CSCs, having either a tumor suppressive and tumor stimulating effect, respectively.

5.2. UPR, intestinal stem cell and colon CSCs

As mentioned above, ER stress/ UPR activation has been identified as an early signal for inducing differentiation of ISCs in the mouse intestine [43]. It was shown that increasing ER stress by thapsigargin (TG) treatment in intestinal organoids *in vitro* or by conditional genetic depletion of *Grp78 in vivo* leads to differentiation of Lgr5-positive ISCs. PERK-eIF2α activation was required for differentiation, and inactivation of this pathway resulted in enhanced stem cell markers expression. The link between the UPR and onset of oncogenic transformation was illustrated by ER stress being able to suppress the progression of

Adenomatous polyposis coli (*Apc*)-mutated and Wnt hyperactive ISCs [55]. In mice, loss of *Apc* together with conditional intestinal deletion of *Grp78* that causes UPR activation resulted in stem cell loss, likely by differentiation, and repopulation by non-mutated ISCs. Interestingly, in the intestinal epithelium of *Apc-/-Grp78-/-* mice, ß-catenin was detected in the nucleus of cells without showing Wnt hyperactivation, suggesting that UPR activation suppresses Wnt signaling downstream of ß-catenin. A possible explanation was provided by experiments indicating that PERK-eIF2α activation resulted in loss of c-Myc expression, which was shown earlier to suppress nuclear ß-catenin activity [56].

In intestinal epithelial cell-conditional *Xbp1-/-* mice the proliferation of ISCs was enhanced [57]. Hyperproliferation could be linked with a proinflammatory Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)/ Interleukin 6 (IL-6)/ Janus kinase (JAK)/ Signal transducer and activator of transcription 3 (STAT3) activation loop. Furthermore, *Apc-/-Xbp1-/-* mice displayed increased ISC proliferation and intestinal tumor formation in a IRE1-dependent and cell autonomous way, identifying Xbp1 as a repressor of intestinal tumor formation.

In *Drosophila* PERK also was identified as an important regulator of intestinal epithelium homeostasis and regeneration [58]. PERK was found to induce ISC proliferation via both cell autonomous and non-autonomous mechanisms. ER stress in ISCs generates misfolded proteins and elevated ROS levels resulting in cell intrinsic PERK-eIF2α and PERK-Nrf2 activation. Interestingly, ER stress in more distant cells, via JNK-dependent production and secretion of inflammatory cytokines, could also induce PERK activation in ISCs via JAK/STAT signaling, identifying PERK as a STAT substrate. Importantly, upon aging, flies displayed chronic PERK-eIF2α activation resulting in intestinal cell dysplasia and loss of barrier function, which could be counteracted by inducible silencing of PERK expression resulting in extended lifespan.

In patient-derived colon-CSCs UPR activity was also shown to reduce stemness and induce differentiation [59]. Treatment of colon-CSC with subtilase cytotoxin AB (SubAB), a bacterium-derived protease that specifically cleaves ER chaperone GRP78, resulted in UPR activation and decreased stemness in which PERK-eIF2α activation was sufficient. SubAB-induced differentiation was reversible since removal of the drug restored stemness. Interestingly, ER stress-differentiated colon-CSCs were sensitized for platinum-based chemotherapy as shown in *in vitro* and *in vivo* models. The authors proposed that ER stress induced differentiation provides a possible therapeutic approach for treating colon cancer. Further work by the same group showed that doxycycline-inducible overexpression of XBP1s and a constitutive active ATF6 variant in a colon-CSCs resulted in reduced stemness and proliferation accompanied by cell cycle arrest [60]. The effects of active ATF6 and particularly XBP1 were found to be mediated by PERK-eIF2α since inhibition of eIF2α by overexpression of the phosphatase GADD34 rescued growth arrest and prevented loss of stemness.

Overall, several studies found that particularly the PERK-eIF2α branch of the UPR is regulating ISC and colon-CSC proliferation, generally by inducing differentiation leading to stem cell depletion, although stemness enhancing activity was also reported for PERK. XBP1s and ATF6 were found to reduce stemness via PERK, although XBP1 could also reduce stemness in ISCs.

5.3. UPR and glioblastoma stem cells and differentiation

In aggressive adult brain tumors, glioblastoma (GBM), enhanced UPR activity has been reported that was linked to worse prognosis [61]. In GSCs ER stress was demonstrated to reduce stemness and tumor forming potential [62]. Treatment with tunicamycin (TM; N-glycosylation inhibitor) reduced self-renewal and pretreated GSCs showed strongly reduced tumor growth when implanted as subcutaneous or intracranial xenografts. The tumor suppressive effect could be linked with TM triggering apoptosis as well as strong decrease of SOX2 expression, SOX2 being important for maintaining stemness in GSCs because ectopic overexpression of SOX2 rescued loss of self-renewal *in vitro*. Since similar levels of SOX2 mRNA were detected in the polysome fraction of untreated and TM treated cells, reduced SOX2 levels were likely a result translational or post-translational inhibition.

In another study, GSCs were also reported to be sensitive for the cytotoxic effects of ER stress (TM and TG) whereas serum-differentiated counterparts were more resistant [63]. ER stress reduced stemness in several GSCs models and PERK was identified as an important mediator of both cytotoxicity and inhibition of stemness. Similar as reported earlier, ER stress-dependent decreases in SOX2 protein expression were detected, but in addition PERK was found to be instrumental for SOX2 downregulation. Moreover, the differentiation capacity of generated PERK deficient GSCs was impaired as illustrated by inability to reduce SOX2 levels and showed delayed and lower levels of the differentiation marker GFAP. Interestingly, PERK and eIF2α phosphorylation appeared not required for SOX2 downregulation, and a noncanonical PERK pathway was proposed to control SOX2 expression, stemness and differentiation of GSCs.

Whereas the results above indicate a role for PERK pathway in maintaining stemness and mediating differentiation, the IRE1-XBP1 pathway has been reported to maintain a differentiated GBM status [64,65]. In GBM TCGA data sets, a low IRE1 activity gene signature correlated with elevated levels stem cell biomarkers (incl. Oligodendrocyte transcription factor 2 (OLIG2), SOX2) and *vice versa*. This suggested that IRE1 activity, particularly of XBP1s, is involved in a differentiated GBM phenotype. Indeed *in vitro* genetically impaired or pharmacological blocked IRE1 signaling resulted in GBM cells with elevated stem cell properties with increased GSC marker expression and clonogenic activity. Reduced stem cell markers expression was confirmed in a IRE1 deficient GL261 xenografts model. Moreover, IRE1 inhibition hardly affected FCS-induced differentiation of GSCs. Reduced SOX2 levels in differentiated cell was found to be mediated by XBP1s-dependent upregulation of miR148a expression that contains SOX2 mRNA binding sites thus delineating a novel IRE1-dependent mechanism that maintains a differentiated GBM phenotype.

Taken together, the above suggests the interesting possibility that PERK and IRE1 pathways have opposite activity in GSC regulation, PERK being required for maintaining stemness and IRE1 for maintaining a differentiated phenotype, although this hypothesis needs further verification.

6. Therapeutic perspectives

The dependency of cancer cells on UPR mediated cell survival has encouraged the development of UPR directed therapeutic strategies. Depending on the UPR status in cancer cells one approach aims to actively enhance proteotoxicity and trigger UPR-mediated cell death and the other one to target either one the UPR sensors IRE1, PERK or ATF6 and block their proteostasis maintaining abilities to interfere with cell survival [66]. Examples of drugs belonging to the first class (ER stress inducers) include already mentioned TG, TM and SubAB but also chemotherapeutics. Drugs belonging to the second class are small molecules and have been predominantly directed against PERK and IRE1. For example, GSK2606414/ GSK2656157 block the ATP-binding domain of PERK and prevent kinase activation [67], whereas guanabenz, sephin1, salubrinal and ISRIB antagonize the effects of downstream activated eIF2α [68]. Moreover, PERK activators (CCT020312, MK-28) have also been developed [69,70]. Sunitinib, a receptor tyrosine kinase inhibitor, and the bioflavonoid quercetin can inhibit IRE1 kinase activity and A106, 4u8c, MKC-3946 and MKC-8866 inhibit IRE1 RNase activity [71].

These UPR modulatory agents have been used in preclinical studies to examine underlying biology and contribution of the UPR and its individual branches to cancer cell survival as well as their possible use as cancer therapeutics. Currently these UPR modulators have not entered clinical testing, except for MKC-8866 (ORIN1001) in early stage testing in breast cancer patients with advanced tumors in combination with

taxane molecules (https://clinicaltrials.gov/ct2/show/NCT03950570). Clinical use is hampered by reported off-target effects of for example the GSK PERK inhibitors that were reported to also inhibit necroptosis regulator Receptor-interacting serine/threonine-protein kinase (RIPK1) and receptor tyrosine kinase cKIT [72,73]. The dual role of the UPR in regulating tumor cell survival and cell death may complicate the therapeutic use of UPR targeting drugs. Their ability to modulate self-renewal and differentiation of CSCs and therapeutic consequences have been hardly explored. More in depth molecular knowledge of the UPR and its individual branches is required to understand better the consequences of individually targeting PERK, IRE1 or ATF6 branches, in order to identify the best therapeutic approach, which will also depend on the cancer type. Stratification of tumors will be helpful to determine the optimal UPR targeting strategy. For example, in GBM, transcript signatures have been generated associated with low or high IRE1 activity, including differences in XBP1 and RIDD activity, that could be used to predict activity in tumor samples in order to select patients eligible for treatment with IRE1 RNase inhibitors [64].

In summary, the UPR regulates multiple hallmarks of cancer and based on the above this also includes the regulation of stemness and differentiation in CSCs. More work is required to deepen our understanding of the molecular mechanisms that underly UPR-dependent CSCs regulation and the roles of -and interactions between the different branches, particularly of IRE1 and PERK, although the current understudied function of ATF6 in this context should also be addressed.

CRediT authorship contribution statement

Dong Liang: Investigation, Writing - original draft, Visualization. **Mohammad Khoonkari:** Investigation, Visualization. **Tony Avril:** Writing - review & editing, Funding acquisition. **Eric Chevet:** Conceptualization, Writing - review & editing, Funding acquisition. **Frank A.E. Kruyt:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

EC is founder of Cell stress discoveries Ltd and of Thabor therapeutics.

DL, MK, TA and FAEK: None.

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