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## When Endoplasmic Reticulum Proteostasis Meets the DNA Damage Response

M. González-Quiroz, A. Blondel, A. Sagredo, C Hetz, E. Chevet, R Pedeux

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2 When Endoplasmic Reticulum Proteostasis Meets the DNA-Damage Response

3

4 Authors: Matías González-Quiroz <sup>1,2,3,4,5,\*\*</sup>, Alice Blondel <sup>4,5,\*\*</sup>, Alfredo Sagredo <sup>1,2,3,</sup>

5 Claudio Hetz <sup>1,2,3,6</sup>, Eric Chevet <sup>4,5</sup>, Remy Pedeux <sup>4,5,\*</sup>

6

7 1 Biomedical Neuroscience Institute (BNI), Faculty of Medicine, University of Chile,

8 Santiago, Chile.

9 2 Center for Geroscience, Brain Health and Metabolism (GERO), University of

10 Chile, Santiago, Chile.

11 3 Program of Cellular and Molecular Biology, Institute of Biomedical Sciences,

12 University of Chile, Santiago, Chile.

13 4 INSERM U1242, Chemistry, Oncogenesis, Stress & Signaling Laboratory,

14 Université of Rennes 1, Rennes, France.

15 5 Centre de Lutte contre le Cancer Eugène Marquis, Rennes, France.

16 6 The Buck Institute for Research in Aging, Novato CA 94945, USA.

17 \*\* Equal contribution.

18

19 Twitter: @matiaeduardo, @HetzLab, @Eric\_Chevet, @CSignaling, @InstitutoBNI

20

21 \*Correspondance to R. Pedeux ([remy.pedeux@univ-rennes1.fr](mailto:remy.pedeux@univ-rennes1.fr))

22

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28 ATM

29

30 **Abstract**

31 Sustaining both proteome and genome integrity requires the integration of a wide  
32 range of mechanisms and signaling pathways. The latter comprise, in particular,  
33 the unfolded protein response (UPR) and the DNA damage response (DDR).  
34 These adaptive mechanisms take place respectively in the endoplasmic reticulum  
35 and in the nucleus. Alterations in UPR and DDR are associated with aging and with  
36 the occurrence of pathologies such as degenerative diseases, metabolic and  
37 inflammatory disorders, and cancer. Here we discuss the emerging signaling  
38 crosstalks between the UPR stress sensors and the DDR and their implication in  
39 cancer biology.

40

## 41 **Introduction**

42 Maintenance of protein homeostasis (**proteostasis**) is mediated by a  
43 network of interconnected quality-control processes, ensuring a functional  
44 proteome [1]. Deregulation of endoplasmic reticulum (ER) proteostasis is a  
45 common feature of several metabolic, degenerative, immunological, or neoplastic  
46 diseases [2,3]. ER proteostasis surveillance is mediated by the **unfolded protein**  
47 **response (UPR)**, a signal transduction pathway that senses protein biogenesis  
48 defects in the ER [2]. Likewise, alteration of **genome integrity (GI)** and the  
49 mechanisms involved in GI maintenance, prevent inherent and sporadic genetic  
50 diseases. An evolutionarily conserved mechanism, the **DNA damage response**  
51 **(DDR)** ensures GI through the recognition of DNA lesions, followed by the initiation  
52 of a signaling cascade resulting in DNA repair [4]. Recently, failure in maintaining  
53 GI was associated with ER proteostasis alteration [5–10]. In addition, some studies  
54 now support a fundamental biological function for UPR sensors in the maintenance  
55 of GI and DNA-damage gene expression [11–14].

56 In this review we describe the UPR and DDR sensors, their mechanisms of  
57 action, their impact on global proteostasis and on the activation of the DDR. We  
58 discuss emerging connections between the UPR and the DDR and we focus on  
59 cancer, given the relevance of both pathways as hallmarks of this disease.

60

## 61 **ER Proteostasis and the Unfolded Protein Response**

62 The ER is the gateway to the secretory pathway through which ~30% of the  
63 cellular proteins transit. Proteins acquire proper folding and conformation in the  
64 ER, thus making this compartment a key contributor to cellular proteostasis [1]. ER  
65 proteostasis disruption can be due to i) malfunctions of ER proteostasis control  
66 mechanisms, ii) the accumulation of improperly folded proteins, or iii) the  
67 imbalance between protein folding capacity and demand, and yields a condition  
68 called ER stress.

69 To cope with ER stress, the UPR, a homeostatic signaling pathway that aims  
70 at restoring ER proteostasis, is triggered to increase ER protein folding and  
71 clearance capacity or to promote cell death programs if the stress cannot be

72 resolved (see box 1) [15,16]. Under basal conditions (non stressed), it is believed  
73 that ER stress sensors are maintained inactive through the binding to the ER  
74 luminal chaperone **binding immunoglobulin protein (BiP)**, whereas the  
75 accumulation of misfolded proteins triggers BiP release from the stress sensors  
76 thereby allowing their activation (Figure 1) [17]. The execution of the UPR results in  
77 i) the reduction of misfolded proteins in the ER resulting from the transient  
78 attenuation of mRNA translation; (ii) the improvement of the ER folding capacity by  
79 increasing the expression of ER-resident chaperones proteins; (iii) the  
80 enhancement of ER protein clearance by increasing its degradation capacity (e.g.,  
81 through the **ER-associated degradation (ERAD)**[18]); (iv) the enhancement of the  
82 export capacity (e.g. through the upregulation of the expression of several genes  
83 whose products are involved in ER cargo exit) (Figure 1) [19].

84 The UPR is transduced by three ER-resident proteins, **IRE1 $\alpha$** , **PERK** and  
85 **ATF6 $\alpha$** , whose primary function is to activate the signalling pathways whose aim is  
86 to restore ER proteostasis [2]. However, when ER stress cannot be resolved, these  
87 UPR sensors activate death signalling pathways (see Box 1) [1]. IRE1 $\alpha$  is a type I  
88 transmembrane protein, that exhibits kinase and endoribonuclease (RNase)  
89 activities in its cytosolic domain. Under ER stress, IRE1 $\alpha$  oligomerizes, then trans-  
90 autophosphorylates which triggers a conformational change that activates the  
91 RNase domain. IRE1 $\alpha$  RNase activation, together with the tRNA ligase RtcB,  
92 induces the non-conventional splicing of X-box binding protein 1 (XBP1) mRNA,  
93 [2,20–22]. The spliced XBP1 mRNA encodes the transcription factor XBP1s which  
94 promotes the transcription of a number of genes whose products are involved in  
95 the ER proteostasis such as foldases, oxidoreductases and ERAD components  
96 (Figure 1) [2,16]. Alternatively, IRE1 $\alpha$  RNase degrades multiple mRNAs and  
97 miRNAs in a sequence-specific process called regulated IRE1 $\alpha$ -dependent decay  
98 (RIDD) of RNA [23]. Although RIDD activity has been proposed to be necessary for  
99 the maintenance of ER homeostasis [24,25] and the pathogenesis of diabetes [26],  
100 cancer [27] and inflammatory conditions [28,29] most of the available evidences  
101 are difficult to interpret due to the concomitant activation of *Xbp1* mRNA splicing  
102 and RIDD activity.

103 PERK is a ubiquitously expressed type I transmembrane serine/threonine  
104 kinase. Under ER stress, PERK oligomerizes and trans-autophosphorylates to  
105 acquire full kinase catalytic activity and to phosphorylate the eukaryotic translation  
106 initiator factor-2 (eIF2 $\alpha$ ), thereby attenuating the general protein synthesis [2,30].  
107 This limits the entry of newly synthesized proteins in the ER while allowing the  
108 selective translation of a growing set of specific mRNA such as that coding for the  
109 activating transcription factor 4 (ATF4), a transcription factor which promotes the  
110 antioxidant response, amino acid metabolism, ER folding capacity, upregulation of  
111 macro-autophagy and therefore has an important pro-survival role (Figure 1) [2].  
112 Additionally, ATF4 expression engages the apoptotic program through the  
113 expression of CHOP protein (also known as GADD153), a transcription factor that  
114 upregulates the pro-apoptotic members of the BCL-2 family and GADD34 (see box  
115 1) [2,31].

116 ATF6 $\alpha$  is a single-pass type II transmembrane protein located in the ER  
117 under resting conditions. ATF6 $\alpha$  bears a bZIP transcription factor on its cytosolic  
118 domain that is released upon ER stress [2,32]. The accumulation of improperly  
119 folded proteins in the ER causes ATF6 $\alpha$  to be exported to the Golgi apparatus and  
120 processed by the S1P and S2P proteases [16]. This process mainly leads to the  
121 release of the cytosolic fragment domain of ATF6 $\alpha$  [16]. In the nucleus, ATF6 $\alpha$   
122 cytosolic domain, simultaneously with XBP1s, upregulates the expression of CHOP  
123 and other genes involved in the regulation of ER size, protein-folding capacity, and  
124 the ERAD (Figure 1) [33,34].

125 Remarkably, reprogramming of UPR signaling has been linked with the  
126 acquisition of several distinctive hallmarks of cancer [35]. Tumour cells are  
127 exposed to several **cell-extrinsic** and **-intrinsic** perturbations that promote the  
128 selective pressure to engage the UPR signalling [19,35]. In general, IRE1 $\alpha$  and  
129 PERK signaling contribute to cancer progression by promoting tumour growth and  
130 cell survival in different type of tumours [27,36–39]. However, there are only few  
131 studies that link ATF6 $\alpha$  activity and cancer. The expression of ATF6 $\alpha$  is elevated in  
132 colorectal cancer but not in normal mucosa [40] and its expression correlates with  
133 a poor prognosis [41]. In human epidermoid carcinoma cells, ATF6 $\alpha$  signaling

134 increases Rheb expression, which in turn activates mTOR signaling (Figure 2) [42].  
135 In addition, protein disulfide isomerase 5 (PDIA5)-dependent activation of ATF6 $\alpha$   
136 was described to be instrumental in the acquisition of Imatinib resistance in chronic  
137 myeloid leukemia (Figure 2) [43]. Although the role of UPR signaling in the tumour  
138 biology is supported by strong evidence [35,39,44], the specific molecular  
139 relationship with the genomic instability has not been studied in depth.

140

### 141 **Genome Integrity and the DNA Damage Response**

142 The preservation of genomic integrity represents a challenge because DNA  
143 is constantly exposed to endogenous and exogenous sources of damage. To  
144 ensure the genome protection, cells have evolved mechanisms for the detection  
145 and repair of DNA lesions called the DDR. The DDR comprises different pathways  
146 that can be triggered either by single-strand breaks (SSB) (e.g., mismatch  
147 mediated repair (MMR), nucleotide excision repair (NER) or base excision repair  
148 (BER)) or by **double-strand breaks (DSB)** (e.g., non-homologous end joining  
149 (NHEJ) and homologous recombination (HR)) [45].

150 DSB are one of the most harmful injuries to the genome [4]. Failure in DSB  
151 repair contributes to the genomic instability that drives cancer development [4,46].  
152 The response to DNA double-strand breaks is controlled by three kinases that are  
153 members of the phosphoinositide 3-kinase (PI3K)-related kinases family: **ATM**,  
154 **ATR**, and **DNA-PKcs** [4]. Those kinases coordinate the phosphorylation of  
155 numerous proteins, ultimately regulating a broad spectrum of cellular processes  
156 such as DNA replication and repair, cell-cycle progression regulation, and  
157 apoptosis or senescence initiation (Figure 1) [47]. Depending on the mechanisms  
158 inducing DSB and the cellular context, different kinase pathways are favored such  
159 as ATM-Chk2 signaling along with the MRE11-RAD50-NBS1 (MRN) complex [4].  
160 ATR-Chk1 is recruited and activated to DSB by single-stranded DNA coated with  
161 replication protein A (RPA) together with its partner ATR-interacting protein  
162 (ATRIP) [4]. The MRN complex is recruited to DNA DSB immediately after its  
163 occurrence, recruiting ATM to the chromatin and stimulating its kinase activity [4].  
164 ATM activates a widespread DSB-signaling cascade that begins with **H2AX**

165 **phosphorylation ( $\gamma$ H2AX).**  $\gamma$ H2AX is a marker of double-strand DNA damage, its  
166 phosphorylation is one of the first response after DSB, and the intensity of the  
167 response is proportional to the number and size of DSB foci [48]. Chk2 is activated  
168 by ATM and redistributed throughout the nucleus where it functions along with  
169 Chk1 in the cell cycle checkpoint signaling network and DNA repair [49]. Chk1  
170 activation after DNA damage is a key function of ATR since activated Chk1 is  
171 essential for S and G2/M phase cell cycle regulation [4]. The activation of Chk1  
172 and Chk2 induces the phosphorylation of the transcription factor p53 and the  
173 subsequent transcription of p53 target genes [50,51]. These cellular mechanisms  
174 are critical in the maintenance of genome integrity and prevention of diseases.

175

### 176 **UPR and DDR signaling in genomic integrity**

177 Maintaining GI is critical to prevent diseases such as cancer, but once  
178 cancer occurs it is important to promote the survival of cancer cells. Genotoxic  
179 stress is key in most of the cancer treatments, because the collapse of DDR  
180 activation and DNA repair mechanisms trigger cell death [52,53]. Interestingly, in  
181 patients with a poor prognosis there is a significant correlation between higher BiP  
182 expression and chemoresistance[19,39]. Moreover, in a panel of cancer cell lines,  
183 genotoxic drugs promote changes in ER structure in a process mediated by the  
184 transcriptional activation of p53 (one of the main tumour suppressor and a key  
185 player in DDR (see box 1)), which generates the expression of receptor  
186 expression-enhancing protein 1 and 2 (REEP1/2) and p53-induced gene 8 (PIG8),  
187 three ER-shaping proteins [54].

188 To date few evidences, connect the UPR and SSB repair. Among them,  
189 HMGB1, a protein mainly involved in MMR and BER was shown to be associated  
190 with the UPR after ER stress in Huntington's disease. Nonetheless, this link was  
191 revealed using a bioinformatic analysis and thus should be carefully considered  
192 and confirmed experimentally [55]. In addition, the key BER protein, APEX1, was  
193 shown to be induced at the transcriptional level by ER stress in human hepatoma  
194 cancer cells [56]. Lastly, ChIP-seq experiments revealed that XBP1s binds to the  
195 promoter region of several BER, MMR and NER genes [12,57]. In summary, the



196 links between UPR and SSB are mainly due to transcriptional control of DDR  
197 genes (*BRCA1, FEN1, H2afx, XRCC1, XRCC4, Parp1, Mre11a and Rad51*) by  
198 UPR sensors or ER stress stimuli (Figure 2). Aside from this compilation of  
199 evidences, most of the links are found to be established upon DSB repair,  
200 highlighting the important role of this mechanism in the DDR and UPR  
201 proteostasis.

202         Recently, in human osteosarcoma cells, ATM was shown to differentially  
203 regulate proteostasis under DNA damage conditions and **oxidative stress**.  
204 Oxidative stress is a natural biological process to which all cells are subjected, and  
205 that is recurrent among different pathologies such as degenerative, metabolic,  
206 immunological diseases, or cancer [58]. In *C. elegans*, it has been observed that  
207 the collapse of proteostasis, associated with the accumulation and aggregation of  
208 misfolded proteins, is directly associated with oxidative stress characteristic of  
209 aging [59]. Indeed, the expression of mutated forms of ATM resistant to oxidative  
210 stress has a slight effect on the DDR, but favors the clearance of toxic protein  
211 aggregates [6]. The loss of ATM function under oxidative damage causes wide  
212 cellular stress as ATM functions are not limited to its participation in DNA-repair or  
213 a specific cellular compartment [6,60]. It is possible to speculate that in  
214 physiological conditions, ATM plays a role as an oxidative stress sensor,  
215 additionally sensing alterations in other cell compartments, including the ER  
216 [10,61]. Accordingly, in *S. cerevisiae*, the ATM/ATR ortholog, Mec1, is a key  
217 component of the signaling network promoting survival in response to proteotoxic  
218 stress [62]. Mec1 regulates the expression of genes linked to proteostasis, and its  
219 inactivation leads to widespread protein aggregation and cell death [62].  
220 Interestingly, protein aggregation is resolved by the activation of autophagy, which  
221 facilitates aggregate clearance [62]. Similarly, in mouse fibroblasts, inhibition of  
222 chaperone-mediated autophagy leads to hyperphosphorylation and destabilization  
223 of the MRN complex and regulated degradation of Chk1 protein [63]. This suggests  
224 that autophagy may contribute to GI by ensuring nuclear proteostasis (Figure 1). It  
225 has been demonstrated that alterations in the functionality of DDR proteins, e.g.

226 ATM, correlate with several pathologies other than cancer, such as  
227 neurodegenerative syndromes [64,65] or systemic autoimmune diseases [66–68].

228

### 229 **The UPR sensors in the DNA damage response**

230 Several studies have reported functional links between UPR and DDR  
231 signaling. Below, we detail the most recent studies in the field, mainly associated  
232 with the role of the IRE1 $\alpha$  and PERK sensors.

233

#### 234 IRE1 $\alpha$ signaling

235 In *S. cerevisiae*, exogenous expression of mammalian XBP1 was found to play  
236 a role in NHEJ DSB repair pathway through the regulation of H4 acetylation [69].  
237 Initially, the regulatory network governed by XBP1s was studied in mouse cells  
238 from plasma, pancreatic  $\beta$ , and skeletal myotubes cells, revealing that XBP1s  
239 regulates the transcription of a cluster of DNA-repair genes under ER stress [57].  
240 Similar experiments were performed in human hepatic cells confirming that XBP1s  
241 directly controls the transcription of multiple DDR genes and the levels of  $\gamma$ H2AX  
242 (Figure 2) [12]. Moreover, silencing of XBP1s causes an increase in the formation  
243 of  $\gamma$ H2AX foci as well as a reduction in the expression of MRN complex proteins  
244 and in ATM phosphorylation (Figure 2) suggesting an increase in DNA damage  
245 coupled with a reduction in damage recognition and processing [70]. XBP1s is not  
246 only involved in the regulation of DDR genes but is also directly linked to genotoxic  
247 stress response (Figure 2). In human oropharyngeal carcinoma cells, UV-radiation  
248 increases the phosphorylation of IRE1 $\alpha$  and the expression of XBP1s, thereby  
249 triggering an increase in interleukin-6 expression [70]. In addition, in human  
250 colorectal cancer cells, exposure to genotoxic drugs, such as doxorubicin and 5-  
251 fluorouracil, was found to reduce IRE1 $\alpha$  expression and XBP1 mRNA splicing in a  
252 p53-dependent manner [71]. However, those results should be taken with caution  
253 as the genotoxic stress-induced phenotypes are diverse, and the responses  
254 depend on the cell type and agent used. XBP1s regulates the expression of Cul5-  
255 ASB11, a ubiquitin ligase targeting BIK, a pro-apoptotic protein [71]. A decreased  
256 XBP1s expression reduces Cul5-ASB11 level and increases the expression of BIK

257 protein under DNA damage. This leads to increased apoptosis, whereas apoptosis  
258 is prevented under ER stress [71]. Consequently, ubiquitination and degradation of  
259 BIK regulates cell fate in the opposite way, depending on the stress conditions  
260 (Figure 2) [71]. It is important to note that in human multiple myeloma cell lines,  
261 doxorubicin has been proposed as a pharmacological inhibitor of IRE1 $\alpha$ , since it  
262 reduces XBP1 mRNA splicing and RIDD activity which in turns leads to a decrease  
263 in cell survival [72,73]. This could extend the hypothesis that some genotoxic drugs  
264 could also act as pharmacological inhibitors of IRE1 $\alpha$  in cells such as multiple  
265 myeloma [72], colorectal cancer [71] and triple-negative breast cancer [74].

266 The activity of UPR stress sensors can be regulated by their binding to co-  
267 factors (activators and inhibitors), in addition to post-translational modifications  
268 [16]. The concept of the UPRosome emerged to visualize UPR stress sensors as  
269 platforms onto which different components assemble to generate composite  
270 signals, but also to crosstalk with other signaling pathways to regulate various  
271 cellular processes [75]. An example of these multiple interactions is provided by  
272 fortilin, a pro-survival molecule that through p53, inhibits ER stress-induced cell  
273 death [76]. Fortilin directly interacts with IRE1 $\alpha$ , inhibiting its kinase and  
274 endoribonuclease domains [76]. Moreover, fortilin silencing increases the  
275 expression of XBP1s, which is associated with increased DNA fragmentation and  
276 apoptosis *in vivo*. This suggests that the expression of XBP1s increases the  
277 signaling of DNA damage, modulating the expression of DNA-repair genes (Figure  
278 2) [57,76].

279 Recently, a novel IRE1 $\alpha$  function associated to the decay of mRNA coding  
280 proteins involved in DDR has been identified. In mouse embryonic fibroblasts,  
281 genotoxic drugs exclusively engaged the RIDD activity in the absence of XBP1  
282 mRNA splicing [11]. In this model, IRE1 $\alpha$  deficiency impairs the ability to repair the  
283 genome under DNA damage, disrupting the cell cycle control and the  
284 phosphorylation of checkpoint kinases and the histone H2AX [11]. At the molecular  
285 level, DNA damage triggers the activation of the c-Abl tyrosine kinase, which  
286 operates as a scaffold protein to stabilize IRE1 $\alpha$  oligomers and to favor RIDD  
287 activity [11]. The role IRE1 $\alpha$  activity in controlling the DDR through RIDD was also

288 validated in fly and mouse models, highlighting a relevant role of this UPR  
289 signaling branch in sustaining cell survival and DNA repair under genotoxic stress  
290 [11]. In addition, RIDD activity may play protective roles in glioblastoma, and in an  
291 *in vitro* assay some DNA-damage proteins could be cleaved by endonucleases  
292 domains of IRE1 $\alpha$  [27]. These studies highlight the new findings around the RNase  
293 activity of IRE1 $\alpha$  and its role in pathological conditions, where XBP1s promotes cell  
294 death while RIDD enables cell survival [27].

295

## 296 PERK signaling

297 A direct molecular relationship between PERK and GI is not well  
298 documented. However, the activation of PERK-p-eIF2 $\alpha$ -ATF4 signaling by single-  
299 strand breaks (SSB) was recently described to support cell survival under nutrient-  
300 restricted conditions [77]. As the PERK-NRF2 branch contributes to the  
301 transcriptional regulation of several genes that mediate the antioxidant response,  
302 its alteration has been associated with an increase of ROS after ER stress and the  
303 accumulation of oxidative DNA lesions [78]. The downregulation of PERK in human  
304 breast cancer cells correlates with an increase in the global phosphorylation of  
305 ATM as well as the phosphorylation of its downstream effector Chk2, leading to an  
306 increase in  $\gamma$ H2AX (Figure 2) [79]. It is interesting to highlight that, as discussed in  
307 the previous section, XBP1s downregulation causes an increase in the  $\gamma$ H2AX foci  
308 but triggers a reduction in ATM phosphorylation [70]. The loss of PERK triggers a  
309 significant attenuation of tumour cell proliferation by increased oxidative DNA  
310 damage, leading to G2/M cell cycle checkpoint activation [79]. Under ER stress,  
311 PERK activation has been shown to operate as a negative regulator of DNA  
312 replication in the absence of DNA damage markers by the phosphorylation of the  
313 adaptor protein Claspin and the activation of Chk1 [14]. The suppression of the  
314 general protein translation by eIF2 $\alpha$  phosphorylation reduces cyclin D1 synthesis,  
315 generating an impaired activity of cyclin D1-CDK4 complex followed by an  
316 inhibition of CDK2, hence ensuring cell cycle arrest at G1 phase (Figure 2) [80].  
317 Under ER stress, PERK activity induces the expression of p47, a truncated p53  
318 isoform, which in turn triggers the upregulation of 14-3-3 $\sigma$  proteins that target the

319 phosphatase CDC25. This prevents the activation of the cyclin B/CDK1 complex  
320 and promotes G2/M arrest, facilitating the ER stress resolution by acting in  
321 conjunction with PERK to repress protein synthesis and the ER protein load  
322 [81,82]. Interestingly, PERK signaling promotes radio-resistance in human breast  
323 and lung cancer cells by increasing the DSB repair signaling [83,84] and  
324 chemoresistance in human colon cancer cells by the PERK/NRF2/MRP1 axis [85].  
325 These data open the possibility that PERK inhibitors could be potentially used as a  
326 chemo-sensitization treatment [85].

327

### 328 ATF6 $\alpha$ Signaling

329 In a human breast cancer cell model the expression of mutant p53 was  
330 shown to enhance the pro-survival activity of ATF6 $\alpha$  and to inhibit both IRE1 $\alpha$  and  
331 PERK branches, damping the activation of CHOP and c-Jun N-terminal kinases  
332 (JNK) [13]. This selective activation is necessary for the invasion, migration and  
333 cell survival [13]. Furthermore, p53-mutants exhibit increased ATF6 $\alpha$  activity  
334 (Figure 2) [13]. In addition, ATF6 $\alpha$  expression and the engagement of senescence  
335 have been described in cells subjected to oncogene activation or to UV-irradiation  
336 [86]. Finally, ATF6 $\alpha$  expression was shown to contribute to radio-resistance in  
337 glioblastoma cells through the upregulation of BiP expression (Figure 2) [87].  
338 Nevertheless, the signaling crosstalk between ATF6 $\alpha$  and the DDR and its  
339 regulation is not fully characterized, and experimental results are needed to  
340 broaden the contribution of this UPR branch to the control, regulation and  
341 interaction with the machinery in charge of the genome stability.

342 Most of the reports available to date focus on the role of IRE1 $\alpha$  and PERK  
343 as regulators of the transcription of genes coding for DNA damage proteins, which  
344 in turn modulate processes such as cell cycle progression and apoptosis  
345 engagement impacting the cell fate. The resulting information is pointing towards a  
346 direct molecular interconnection between ER proteostasis and DNA damage  
347 surveillance as a new and exciting research field where these two homeostatic  
348 signaling exert reciprocal and bilateral regulations.

349

## 350 **Concluding Remarks**

351           Recently, several reports have pointed towards interactions between the  
352 UPR and the DDR. This suggests a relationship between the ER stress signaling  
353 and DNA damage and repair pathways, but the significance of these observations  
354 on disease onset is unknown and important questions remain unknown (see  
355 Outstanding Questions). Furthermore, new insights about the role of UPR sensors  
356 in the maintenance of GI, opens up new perspectives regarding therapeutic  
357 targets. Recently, a library of chemotherapeutics compound (> 80 compounds) was  
358 shown to induce immunogenic cell death through PERK and IRE1 $\alpha$  activation.  
359 Future research would be necessary to evaluate more UPR-activation markers in  
360 cancer cells [88] after exposure to radiotherapy or chemotherapeutic drugs such as  
361 etoposide, doxorubicin, oxaliplatin, paclitaxel or temozolomide; considering the  
362 extensive use of these drugs as standard of care in tumour. Moreover, the  
363 administration of these drugs is associated with the activation of IRE1 $\alpha$  in some  
364 models [11,27,72,89,90]. Finally, it is necessary to explore new models to study  
365 UPR and the chemotherapeutic response in cancer. Currently, solid tumours  
366 represent a suitable model to study the effect of UPR activation in cancer. These  
367 types of models have often been useful to reach relevant conclusions about the  
368 UPR in cancer, but the inability to correlate with the interaction with extrinsic  
369 factors such as metabolic stress, hypoxia, or drug availability will always be a  
370 limitation. Furthermore, it would be interesting to study UPR and cancer in  
371 suspended or anchor-free cell models. In these models, the current work correlates  
372 UPR and its effects on the immune response [91–93], leaving aside the role of  
373 chemotherapy or radiotherapy. Thus, the use of anchor-free cancer cells and the  
374 generation of UPR sensors-knockout cells eliminates extrinsic factors. However,  
375 limitations in anchor-free cell models are related to the expression of surface  
376 antigens or the damage-associated molecular patterns (DAMPs) that could be  
377 regulated by the UPR under DNA-damage conditions.

378           The identification of a fundamental biological function for mRNA decay in the  
379 maintenance of genome integrity represents a unique example for selective and  
380 specific activation of RIDD activity with clear physiological implications.

381 Remarkably, IRE1 $\alpha$  is frequently affected by loss-of-function mutations in various  
382 type of cancer [39], contrasting with the notion that cancer cells require IRE1 $\alpha$  to  
383 survive in hypoxic conditions [35,92]. We speculate that the genetic alterations of  
384 IRE1 $\alpha$  observed in cancer may synergize with oncogenes to promote genomic  
385 instability. Overall, a direct inter-connection is emerging between the pathways that  
386 ensure the integrity of the proteome and the genome. It is necessary to explore in  
387 depth how unfolded protein response regulates the gene expression, ribosome  
388 profile and proteins expression in a context of genotoxic stress, using different  
389 multi-omics strategies [94,95], in order to evaluate the global modulation of the  
390 DNA damage response. As evidenced in this review, the crosstalk between UPR  
391 and DDR is of great interest in the context of UPR biology, especially but not  
392 exclusively in cancer biology and treatment.

393

394 **BOX1**

395 **A deadly relationship: Crosstalk between UPR-DDR and their involvement in**  
396 **apoptosis**

397 In response to unresolved DNA-damage and ER stress the apoptotic  
398 program is mainly orchestrated by p53 and CHOP. Both transcription factors are  
399 upregulated as a mechanism to monitor the integrity/stability of the genome and  
400 proteome. CHOP is upregulated by the UPR arms [96] and it is directly related to  
401 the ER stress-induced apoptosis. However, CHOP-deficient cells still undergo  
402 apoptosis, suggesting unknown pro-death signaling. CHOP promotes apoptosis  
403 through the repression of anti-apoptotic and induction of pro-apoptotic genes, such  
404 as, BAD, BIM, NOXA, PUMA and DR5 [97,98]. p53 expression is induced by  
405 several stress signals such as DNA damage and oncogene activation [99].  
406 Moreover, p53 has several non-transcriptional functions [100,101]. Senescence,  
407 cell-cycle arrest, and apoptosis are the most prominent outcomes of p53 [102].  
408 Principally, p53 engages the apoptosis through the transcriptional regulation of the  
409 pro-apoptotic proteins PUMA, BIM, NOXA, and extrinsic apoptotic pathway  
410 components [103]. Interestingly, the crosstalk between these two transcription  
411 factors has been described. CHOP drives the MDM2 expression, promoting p53  
412 degradation [104]. It has been linked to the function and localization of p53 as a  
413 component of the ER stress-induced apoptotic pathway. ER stress promotes p53  
414 expression through NF- $\kappa$ B [105] and CHOP co-operates with FOXO3a to regulate  
415 the expression of PUMA and BIM under ER stress [44,106]. Moreover, p53 is an  
416 important mediator of ER stress–dependent apoptosis through the upregulation of  
417 PUMA [107]. PERK activation modifies the translation of the p53 mRNA from the  
418 full-length to the p53 $\Delta$ N40 (p53/47) isoform and actively suppresses the p21  
419 expression during ER stress, promoting G2 cell-cycle [108]. During chronic ER  
420 stress, p53 induces BIK expression while at the same time suppressing BiP  
421 translation, leading to the dissociation of the BIK/BiP complex and the apoptosis  
422 activation [109]. P53 is located at ER/mitochondria-associated membranes (MAMs)  
423 contact sites modulating the Ca<sup>2+</sup> transfer to the mitochondria [110]. Moreover, p53  
424 regulates autophagy, by the proper localization of PML protein at ER/MAMs [111].



425 Also, PERK and IRE1 $\alpha$  have been identified as components of the ER/MAMs  
426 [112,113], suggesting novel interactions between the UPR-sensors and p53.  
427 Finally, cancer cells are exposed to several factors that alter proteostasis. To cope  
428 with this, tumour cells engage the UPR to manage these disturbances [35]. As p53  
429 mutations are the most recurrent alterations in cancer, leading to the resistance to  
430 stressors as DNA damage, the selective inhibition of pro-survival UPR represent a  
431 promising intervention on p53-deficient tumours, engaging apoptosis by the  
432 induction of unresolved ER stress.

433

#### 434 **Glossary:**

435 **Proteostasis:** Is a network of interconnected quality-control processes in the cell  
436 that maintains a functional proteome. Chaperones, foldases, oxidoreductases and  
437 glycosylating enzymes ensure that secretory proteins are properly folded, modified  
438 and assembled into multi-protein complexes in the ER before they transit further  
439 downstream in the secretory pathway.

440

441 **Unfolded Protein Response (UPR):** Is a signal transduction pathway that senses  
442 the fidelity of protein folding in the ER lumen. The UPR transmits information about  
443 protein folding status to the nucleus and cytosol to adjust the protein folding  
444 capacity of the cell. The UPR is transduced by three principal ER-resident proteins:  
445 inositol-requiring protein 1 $\alpha$  (**IRE1 $\alpha$** ), PKR-like ER kinase (**PERK**), and activating  
446 transcription factor 6 $\alpha$  (**ATF6 $\alpha$** ).

447

448 **Genomic Instability (GI):** Includes all processes that maintain the integrity of DNA  
449 such as sensing, signaling and repair of DNA damage, processing of DNA damage  
450 in the context of chromatin and chromosomes, cell cycle checkpoint control and  
451 apoptosis control. Effective maintenance of genome integrity is essential for  
452 healthy organisms, aging, and prevention of diseases.

453

454 **DNA damage response (DDR):** Cellular response involving DNA damage  
455 recognition, followed by the initiation of a cellular signaling cascade that promotes

456 DNA repair, which can modulate cell-cycle progression, chromatin structure and  
457 transcription, both at sites of DNA damage and globally. DDR after DNA-double  
458 strand breaks is controlled by three related kinases: Ataxia- telangiectasia mutated  
459 (**ATM**), ATM and Rad3-Related (**ATR**) and DNA-dependent protein kinase (**DNA-**  
460 **PKcs**).

461

462 **Binding immunoglobulin protein (BiP):** Is a key ER chaperone and master  
463 regulator of ER functions under ER stress. Detection of misfolded protein species  
464 by the three UPR sensors is partly dependent on BiP.

465

466 **ER-associated degradation (ERAD):** Is the principal quality-control mechanism  
467 responsible for targeting misfolded ER proteins for cytosolic degradation. ERAD  
468 targets are destroyed by the cytoplasmic ubiquitin–proteasome system. Many ER  
469 chaperones participate into the ERAD complex, including BiP, EDEM1, OS9, and  
470 XTP3B. The UPR sensor IRE1 $\alpha$  and SEL1L- HRD1 complexes are the two most  
471 conserved branches of ER quality-control mechanisms.

472

473 **Cell-extrinsic factor:** Any factor that is independent of the genetic background or  
474 alteration of DNA, such as hypoxia, glucose deprivation, or inadequate amino acid  
475 supplies.

476

477 **Cell-intrinsic factor:** Any factor that is dependent on the genetic background or  
478 DNA, such as oncogenic activation, alteration in chromosome number or  
479 hyperploid.

480

481 **DNA double-strand breaks (DSB):** Different classes of DNA damage such as  
482 ultraviolet (UV) light, irradiation, DNA-damage drugs or oxidative stress that leads  
483 to DNA rupture in both strands. If DNA is not repaired correctly, DSB can cause  
484 deletions, translocations, and fusions of the DNA.

485

486 **Gamma-H2AX ( $\gamma$ H2AX):** Upon DSB induction, the histone variant H2AX is  
487 phosphorylated on serine 139 by ATM, ATR or DNA-PK, generating  
488 phosphorylated H2AX, or so-called  $\gamma$ H2AX.  $\gamma$ H2AX induction is one of the earliest  
489 events detected in cells and human biopsies following exposure to DNA damaging  
490 agents.  $\gamma$ H2AX is a key marker of double-strand DNA damages, allowing the  
491 activation and relocalization of repair proteins to DSB sites as well as the signal  
492 amplification.

493

494 **Oxidative stress:** Is an imbalance between the production of reactive oxygen  
495 species (free radicals) and antioxidant defenses. Amino acids such as proline,  
496 arginine, lysine and threonine are particularly vulnerable to oxidative damage, both  
497 as free molecules or within proteins. Moreover, oxidative damage can also affect  
498 the integrity and stability of DNA and RNA.

## Figure Legend.

**Figure 1. Unfolded Protein Response and DNA Damage Sensors.** All three ER stress sensors (PERK, IRE1 $\alpha$ , ATF6) are localized at the ER membrane and under ER stress they activate signaling events that increase protein-folding capacity and reduce protein load on the ER. In response to DNA damage, ATM is activated and recruited to DSBs by the MRE11-RAD50-NBS1 complex and ATR is recruited to RPA-coated ssDNA by its binding partner ATRIP. DNA-PKcs, meanwhile, is recruited and activated by Ku-bound DSB ends. The UPR transcription factors and DNA damage proteins determine cell fate by the regulation of distinct subsets of target genes spanning from the recovery of ER homeostasis to DNA damage response. The green boxes illustrate the common target and functions induced by the ER stress and genotoxic stress. The blue boxes illustrate UPR functions induced by the ER stress response. The grey box illustrates the DDR proteins involved in the UPR. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License.

**Figure 2: Unfolded Protein Response sensors involved in the DNA Damage Response.** IRE1 $\alpha$  is maintained in a repressed state through an association with BiP. Upon ER or genotoxic stress, misfolded proteins dock to BiP, thus triggering the dissociation from IRE1 $\alpha$ . This triggers IRE1 $\alpha$  dimerization and auto-transphosphorylation inducing its activation. IRE1 $\alpha$  may also bind misfolded proteins to oligomerize. IRE1 $\alpha$  allows the splicing of XBP1 mRNA and the degradation of RNAs (RIDD). XBP1s transcriptional factor governs notably the expression of genes involved in DDR and ubiquitin ligases. RIDD activity governs the expression of mRNA, which impact on DDR proteins. IRE1 $\alpha$  activity can be modulated by fortilin, c-Abl, p53, doxorubicin or 5-fluorouracil. Knockdown of XBP1 reduces ATM phosphorylation, MRN complex expression and increases  $\gamma$ H2AX. The mechanism of PERK repression and activation is the same as for IRE1 $\alpha$ . Activated PERK phosphorylates eIF2 $\alpha$ , which in turn shuts down global translation and concomitantly increases the expression of the transcription factor ATF4. The

stopping of global translation impact on Rad51, p47 and p53 along with inhibiting cyclin D1 expression, ultimately dysregulating G1 and G2/M cell cycle phases. ATF4 transcriptional factor bind CHOP itself inducing GADD34 transcription that creates a feedback mechanism. PERK silencing increases p-ATM thus triggering  $\gamma$ H2AX and Chk2 activation, impacting on cell cycle. ATF6 is exported from the ER to the Golgi where it is cleaved by S1P and S2P proteases, allowing the release of its cytosolic domain, which is a potent transcription factor named ATF6f. PDIA5 and mutant-p53 increase ATF6 activity, promoting Imatinib resistance in cancer and allowing RHEB expression and mTOR signaling. Ub: ubiquitination; P: phosphorylation. This figure was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License.

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## Highlights

- Alteration in the genome integrity has been associated with disruption of the endoplasmic reticulum (ER) proteostasis
- The unfolded protein response (UPR) and the DNA damage response (DDR) play important roles in the development and progression of several diseases, including cancer.
- UPR sensors IRE1 $\alpha$ , PERK and ATF6 $\alpha$  play a role in response to genotoxic and ER stress in cells by interacting with DNA damage proteins functions (e.g. ATM, ATR, p53, p21, Chk1 or Chk2).
- The crosstalk between UPR and DDR may contribute to cancer progression. Indeed, CHOP and p53 play a central role in the crosstalk between UPR and DDR.
- The pharmacologic modulation of the UPR could enhance the chemotherapy and radiotherapy effectiveness

## Outstanding Questions

- Do DDR associated proteins participate in the UPR signaling and regulation?
- Could the UPR impact the chemotherapy or radiotherapy-induced genotoxic stress and therefore modulate the response to cancer treatment?
- Post-translational modifications such as ubiquitination are critical in DDR signaling. Can UPR interfere with DDR proteins stability by modulating post-translational modifications?
- What are the most relevant cancer models that can be used to study UPR and its effect on DNA damage response?



