

Targeting unfolded protein response in cancer and diabetes

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Abstract

The maturation of secretory and membrane proteins in the endoplasmic reticulum (ER) is tightly regulated by the unfolded protein response (UPR), a signal transduction pathway maintaining ER protein folding homeostasis. However, certain ER states are incompatible with cell survival and therefore the UPR may choose to eliminate severely disrupted cells by apoptosis. This is accomplished primarily through the activation of the transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP). In the April 2015 issue of *Endocrine-Related Cancer*, researchers from the universities of South Carolina and Athens (Greece) suggested a novel mechanism of CHOP-mediated apoptosis connected with the suppression of a prominent cell cycle regulator with anti-apoptotic activity, p21. These findings and suggested clinical applications, such as potentiation of cancer chemotherapy and a novel therapeutic approach for type 2 diabetes, are discussed in the context of UPR.

Key Words

- ▶ chemotherapy
- ▶ islet cells
- ▶ oncology
- ▶ intracellular signaling

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The endoplasmic reticulum (ER) of mammalian cells is a complex protein factory where the proteins destined for secretion and cell membranes are folded into their unique three-dimensional structures. This is aided by molecular chaperones, and many secretory proteins are also glycosylated. In addition, ER is also a major depot for intracellular Ca^{2+} . Protein folding is under the constant surveillance of an elaborate quality control system that permits only correctly folded molecules to travel to their cellular or extracellular destinations.

The physiological or pathophysiological conditions that interfere with protein folding, glycosylation or calcium homeostasis, most often lead to the excessive accumulation of unfolded proteins causing so-called ER stress. In response to this, cells have developed a plethora of adaptive reactions aimed at maintaining ER protein folding homeostasis, which are collectively known as the unfolded protein response (UPR) (Brewer 2014).

UPR is composed of three branches, each triggered by one specific transmembrane sensor: ATF6, IRE1, or PERK. The inhibition of the translation initiation factor eIF-2 via its phosphorylation by PERK occurs at the initial stages of UPR, resulting in the curtailing of cellular protein synthesis and subsequent reduction in ER overload by the newly synthesized polypeptides. Further adaptive reactions include activation of ATF6 and IRE1, which lead to the transcriptional upregulation of the genes of molecular chaperones and other helper proteins that boost the folding capacity of the ER. These concerted efforts to restore the ER homeostasis represent an essential pro-survival function of UPR. However, certain ER states may be incompatible with cell survival and therefore UPR switches later to its second function, namely the elimination of severely disrupted cells in an orderly manner by apoptosis. All three of the UPR branches can contribute to the initiation of apoptosis, though to a different extent.

The central role belongs to the IRE1 and PERK-dependent pathways. IRE1 via the activation of JUN N-terminal kinase reduces the activity of anti-apoptotic BCL2 family members (Dhanasekaran & Reddy 2008). It has also been shown that IRE1 can induce selective cleavage of a number of miRNAs that limit translation of key caspases involved in apoptosis (Upton *et al.* 2008).

PERK, despite generally suppressing mRNA translation, paradoxically enough can initiate concomitant selective translation of a few mRNAs including transcription factor ATF4. The latter is important for the activation of a number of pro-adaptive genes; however, one of its downstream targets is yet another transcription factor, CCAAT-enhancer-binding protein homologous protein (CHOP) (Ma *et al.* 2002), which can facilitate cell demise in a number of ways. CHOP has been implicated in the downregulation of the anti-apoptotic factor BCL2 and in increased expression of pro-apoptotic BIM (McCullough *et al.* 2001). In addition, CHOP, together with PERK, can intensify protein synthesis, thereby causing oxidative stress and subsequent cell death (Han *et al.* 2013). The pro-apoptotic mechanisms triggered by CHOP expression are rather complex and are not limited to those described above.

In the April 2015 issue of *Endocrine-Related Cancer*, researchers from the universities of South Carolina and Athens (Greece) present fresh data (Mihailidou *et al.* 2015a,b) describing a novel CHOP-mediated apoptosis mechanism connected with the suppression of a prominent cell cycle regulator, p21/waf1 with a strong pro-apoptotic activity. p21 is an inhibitor of cyclin-dependent kinases and its expression is regulated transcriptionally by the p53 tumor suppressor in response to a variety of stress conditions. Such a dual role of p21 in the regulation of both cell cycle and susceptibility to apoptosis has previously been suggested to be important in the transition of UPR from the pro-survival to pro-apoptotic pathway (Mihailidou *et al.* 2014).

Indeed, as is demonstrated in the first publication (Mihailidou *et al.* 2015a), prolonged UPR downregulates the expression of p21, making the apoptotic scenario more likely. This is supported by a wealth of *in vitro* and *in vivo* data. Tunicamycin, an inhibitor of protein glycosylation in the ER, and therefore a strong UPR inducer when administered intraperitoneally to mice provoked a strong UPR, as expected, which is judged by the overexpression of CHOP and also of the molecular chaperones BiP and GRP94. At the same time, the levels of p21 in several organs were found to be strongly

downregulated. A similar effect was seen in cultured mouse embryonic fibroblasts.

What could be the purpose of UPR-dependent regulation of p21? Is it a novel independent pro-apoptotic pathway, or a previously uncharacterised pleiotropic downstream effect of the ATF4-CHOP axis? As it was suggested previously by the same group, the second hypothesis sounds more plausible as p21 expression seems to be governed by CHOP (Mihailidou *et al.* 2010). This was demonstrated by the absence of p21 downregulation in the tunicamycin-induced CHOP KO mice and also by the drop in p21 mRNA and protein levels in the CHOP-transfected lung cancer cells. As CHOP is a transcription factor, it was logical to suggest that p21 regulation occurs at the transcriptional level. Indeed, a gene-reporter assay using the p21 promoter fragment cloned upstream of the luciferase gene and transfected into the cultured cells showed substantial activation of this construct (increased luciferase expression) upon tunicamycin treatment. Finally, the physical interaction of CHOP with the p21 promoter was tested by the chromatin immunoprecipitation assay that unambiguously demonstrated formation of CHOP-p21 promoter complexes under the UPR conditions.

As indicated above, p21 is known as one of the primary downstream targets of p53. Could p53 be involved in UPR-driven p21 regulation? The answer to this question as presented in Mihailidou *et al.* (2015a) is negative: i) tunicamycin-induced suppression of p21 remains intact in the tissues from p53-deficient mice, and ii) the fragment of p21 promoter activated by tunicamycin did not contain a p53-binding site.

Furthermore, authors attempted to utilize the discovery of this novel signaling pathway to augment the chemotherapeutic activity of doxorubicin (DOX). Indeed, the cytotoxic potential of DOX was increased upon treatment of MEFs or cancer cells with tunicamycin. The mechanism of this effect is linked to the CHOP-induced downregulation of p21 because sensitivity to DOX was higher in p21 KO MEFs, whereas CHOP ablation in these cells offered resistance. A similar effect was seen in SCID mice bearing tumor xenografts: combined treatment with DOX and tunicamycin significantly inhibited tumor growth.

UPR signaling under physiological conditions is most common in the professional secretory cells, which are characterised by the high load of secretory cargo proteins in the ER. One example is β -cells of the pancreatic Langerhans islets producing and secreting large amounts of insulin in response to glucose fluctuations. The functional

pro-survival UPR is required to secure the flawless folding and secretion of this protein. However, UPR in these cells may also play a negative role as there is growing evidence that the molecular mechanisms of β -cell failure and loss of islet mass during the type II diabetes are primarily connected with the late, pro-apoptotic branch of UPR that is activated due to a variety of diabetogenic conditions: lipotoxicity, glucotoxicity, oxidative stress, amyloid deposition, and others (Back & Kaufman 2012). One of the central actors of this UPR pathway, CHOP, has been already reported to be involved in the pathogenesis of diabetes (Oyadomari *et al.* 2002). However, in Mihailidou *et al.* (2015b) the authors make one further step in demonstrating that the CHOP-induced down-regulation of p21 is the critical event in triggering β -cell apoptosis. This notion is supported by the multiple lines of evidence.

Thus, p21 levels were found to be low under the high (glucotoxicity) and low glucose (deprivation) conditions which cause ER stress and consequently activate UPR in hamster pancreatic insulinoma cells. The stress was relieved when cells were incubated with nutlin-3a, the p21 activator. p21 expression in pancreatic cells was shown to be regulated by UPR (tunicamycin) via the transcriptional suppression by CHOP (gene reporter experiments). Moreover, CHOP acts by competing away the C/EBP transcription factors, which are positive regulators of p21 expression. Interestingly, low-to-moderate UPR induction by tunicamycin leads to the stimulation of p21 expression, whereas intense ER stress suppressed it, confirming that downregulation of p21 is dependent on the switch from pro-survival to pro-apoptotic UPR.

A pro-survival function of p21 in β -cells which was analogous to that demonstrated previously in the context of carcinogenesis (Yoon *et al.* 2012) was confirmed in the pancreatic islets isolated from the w/t and p21 KO mice. The latter were found significantly more sensitive to high glucose levels with a characteristic increase in caspase activation – a clear indication of ongoing apoptosis.

Are these *in vitro* results applicable to the situation *in vivo*? These papers give a positive answer to this question: two animal models of type 2 diabetes were investigated, high-fat diet (high fat/sucrose-sweetened drinking water) and streptozotocin (CTZ)-induced diabetes. In the first model that was applied to both w/t and p21 KO mice the latter was found to be more sensitive to diabetes as demonstrated by high glucose levels, delayed response in glucose homeostasis, and lower insulin levels. As in the *in vitro* experiments, nutlin-3a administration effectively restored glucose homeostasis and improved pancreatic

morphology. Importantly, these changes were observed only in p21-positive animals indicating the specificity of the effect. Similar positive results were achieved when CTZ and high-fat diet-treated w/t animals were administered nutlin-3a.

Rephrasing the famous expression of Kurt Lewin, 'There is nothing more practical than a good theory' (Lewin 1952), it can be suggested that there is nothing more practical than the knowledge of the basic mechanisms of cellular regulation. The authors of these two articles followed the letter and spirit of this expression by applying the discovery of this novel twist in UPR-mediated apoptosis to potentiate the effect of anticancer drugs (Mihailidou *et al.* 2015a), and went on to stimulate p21 expression to prevent damage of pancreatic β -cells in diabetes (Mihailidou *et al.* 2015b).

Of course, implementation of these suggestions into the clinical practice would require many more studies and there are many more questions to answer. For instance, it is known that p21 may act not only as a tumor suppressor but also serve as an oncogene by promoting cell growth and inhibiting apoptosis (Yoon *et al.* 2012). Such an oncogenic function is thought to be associated with the cytoplasmic localization of p21 (Zhou *et al.* 2001). It would be interesting to discriminate between these two opposing functions of p21 in the light of the data presented.

In addition to p21, another member of the CIP/KIP family of cyclin-dependent kinase inhibitor proteins, p27, is similarly active in suppressing cell proliferation (Yoon *et al.* 2012). Is it another downstream target of CHOP and thus, is it involved in the pro-apoptotic branch of UPR?

Tinkering with UPR in order to boost the efficacy of chemotherapy is a nice proof of concept; however, practical implementation would require development of the fine-tuned drugs that could activate specific UPR pathway(s) with high precision.

There are many instances of cancers with down-regulated p21, will UPR activation prove to be an effective method to potentiate chemotherapy?

So, contrary to the well known tautology from Donald Rumsfeld, 'We don't know what we don't know', here we appear (at least to some degree) to know what we don't know, and by addressing the questions set out above, we should be able to get exciting answers about the novel aspects of UPR and possible applications in medicine.

Declaration of interest

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References

- Back SH & Kaufman RJ 2012 Endoplasmic reticulum stress and type 2 diabetes. *Annual Review of Biochemistry* **81** 767–793. (doi:10.1146/annurev-biochem-072909-095555)
- Brewer JW 2014 Regulatory crosstalk within the mammalian unfolded protein response. *Cellular and Molecular Life Sciences* **71** 1067–1079. (doi:10.1007/s00018-013-1490-2)
- Dhanasekaran DN & Reddy EP 2008 JNK signaling in apoptosis. *Oncogene* **27** 6245–6251. (doi:10.1038/onc.2008.301)
- Han J, Back SH, Hur J, Lin YH, Gildersleeve R, Shan J, Yuan CL, Krokowski D, Wang S, Hatzoglou M *et al.* 2013 ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nature Cell Biology* **15** 481–490. (doi:10.1038/ncb2738)
- Lewin K 1951 In *Field theory in social science: selected theoretical papers*. Ed D Cartwright. 346pp. New York, NY, USA: Harper.
- Ma Y, Brewer JW, Diehl JA & Hendershot LM 2002 Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *Journal of Molecular Biology* **318** 1351–1365. (doi:10.1016/S0022-2836(02)00234-6)
- McCullough KD, Martindale JL, Klotz LO, Aw TY & Holbrook NJ 2001 Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. *Molecular and Cellular Biology* **21** 1249–1259. (doi:10.1128/MCB.21.4.1249-1259.2001)
- Mihailidou C, Papazian I, Papavassiliou AG & Kiaris H 2010 CHOP-dependent regulation of p21/waf1 during ER stress. *Cellular Physiology and Biochemistry* **25** 761–766. (doi:10.1159/000315096)
- Mihailidou C, Papavassiliou AG & Kiaris H 2014 A crosstalk between p21 and UPR-induced transcription factor C/EBP homologous protein (CHOP) linked to type 2 diabetes. *Biochimie* **99** 19–27. (doi:10.1016/j.biochi.2013.11.003)
- Mihailidou C, Chatzistamou I, Papavassiliou A & Kiaris H 2015a Improvement of chemotherapeutic drug efficacy by endoplasmic reticulum stress. *Endocrine-Related Cancer* **22** 229–238. (doi:10.1530/ERC-15-0019)
- Mihailidou C, Chatzistamou I, Papavassiliou A & Kiaris H 2015b Regulation of p21 during diabetes-associated stress of the endoplasmic reticulum. *Endocrine-Related Cancer* **22** 217–228. (doi:10.1530/ERC-15-0018)
- Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E & Mori M 2002 Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. *Journal of Clinical Investigation* **109** 525–532. (doi:10.1172/JCI0214550)
- Upton JP, Austgen K, Nishino M, Coakley KM, Hagen A, Han D, Papa FR & Oakes SA 2008 Caspase-2 cleavage of BID is a critical apoptotic signal downstream of endoplasmic reticulum stress. *Molecular and Cellular Biology* **28** 3943–3951. (doi:10.1128/MCB.00013-08)
- Yoon MK, Mitrea DM, Ou L & Kriwacki RW 2012 Cell cycle regulation by the intrinsically disordered proteins p21 and p27. *Biochemical Society Transactions* **40** 981–988. (doi:10.1042/BST20120092)
- Zhou BP, Liao Y, Xia W, Spohn B, Lee MH & Hung MC 2001 Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. *Nature Cell Biology* **3** 245–252. (doi:10.1038/35060032)

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