The role of the unfolded protein response in cancer progression: From oncogenesis to chemoresistance

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Tumour cells endure both oncogenic and environmental stresses during cancer progression. Transformed cells must meet increased demands for protein and lipid production needed for rapid proliferation and must adapt to exist in an oxygen- and nutrient-deprived environment. To overcome such challenges, cancer cells exploit intrinsic adaptive mechanisms such as the unfolded protein response (UPR). The UPR is a pro-survival mechanism triggered by accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), a condition referred to as ER stress. IRE1, PERK and ATF6 are three ER anchored transmembrane receptors. Upon induction of ER stress, they signal in a coordinated fashion to re-establish ER homeostasis, thus aiding cell survival. Over the past decade, evidence has emerged supporting a role for the UPR in the establishment and progression of several cancers, including breast cancer, prostate cancer and glioblastoma multiforme. This review discusses our current knowledge of the UPR during oncogenesis, tumour growth, metastasis and chemoresistance.

Introduction

The endoplasmic reticulum (ER) is a major regulator of metabolic processes within the cell, including carbohydrate metabolism, lipid biogenesis and calcium homeostasis. ER chaperones ensure the correct folding of newly synthesised proteins, which are exported following rigorous quality control. Intrinsic and extrinsic cellular stresses, such as depletion of calcium stores, nutrient deprivation, hypoxia and pH alterations [Cubillos-Ruiz et al., 2017], disrupt protein folding efficiency in the ER lumen which leads to the accumulation of misfolded proteins. This phenomenon is known as ER stress.

The unfolded protein response (UPR) is triggered to relieve ER stress and to restore metabolic and protein processing functions. The UPR involves the activation of three ER stress sensors: Inositol Requiring Enzyme 1α (IRE1, also known as ERN1), Protein kinase RNA-activated-like ER Kinase (PERK, also known as EIF2aK3) and Activating Transcription Factor 6 (ATF6). Under homoeostatic conditions, glucose-regulated protein 78 (GRP78), also known as binding immunoglobulin protein (BiP), maintains each of the stress sensors in an inactive conformation. Intrinsic and extrinsic cellular stresses, such as depletion of

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Abbreviations: ATF6, Activating Transcription Factor 6; CSCs, cancer stem cells; DCs, dendritic cells; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERSE, ER stress response elements; eIF2α, eukaryotic translation Initiation Factor 2 subunit alpha; GBM, glioblastoma multiforme; GRP78, glucose-regulated protein 78; HBP, hexosamine biosynthetic pathway; IRE1, Inositol Requiring Enzyme 1; MEFs, mouse embryonic fibroblasts; NRF2, Nuclear Factor Erythroid 2 like 2; O-GlcNAc, uridine diphosphate N-acetylglucosamine; OGT, O-GlcNAc transferase; PERK, Protein kinase RNA-activated-like ER Kinase; RIDD, regulated IRE1 dependent decay; TERS, Transmissible ER Stress; UPR, unfolded protein response; VEGF-A, vascular endothelial growth factor-A; XBP1, X Box binding Protein 1.
et al., 2000; Shen et al., 2002]. However, during ER stress, GRP78 dissociates from each of the sensors, allowing for activation of various adaptive signalling pathways in a collective effort to relieve ER stress. This is achieved by mitigating protein translation to reduce incoming protein load, increasing transcription of ER chaperones to facilitate protein folding, and eliminating misfolded proteins by recycling components of proteins via ER-associated degradation (ERAD) and autophagy [Hetz, 2012]. However, if ER stress persists, the cell switches from an adaptive response to a death-inducing response [Szegezdi et al., 2006; Logue et al., 2013], demonstrating a potential threshold which ER stress must surpass before the cell commits to apoptosis.

Intrinsic stresses in the tumour, such as oncogenic activation, and extrinsic stresses exerted by the tumour environment increase the levels of misfolded proteins in the ER, triggering activation of UPR pathways. The tumour environment is a hypoxic, acidic and nutrient deprived milieu and the three arms of the UPR are highly active in many types of cancer including breast, lung, liver, colorectal and glioma [Shuda et al., 2003; Uramoto et al., 2005; Scriven et al., 2009; Auf et al., 2010; Jin et al., 2016]. Although sustained UPR activation is linked to induction of apoptotic signalling, cancer cells can bypass this apoptotic switch and exploit the UPR to promote proliferation and metastasis. Furthermore, recent evidence indicates that, in addition to aiding tumour progression, UPR activation can also limit the effectiveness of chemotherapy by contributing to the development of chemoresistance [Jiang et al., 2009; Feng et al., 2011; Chen et al., 2017; Salaroglio et al., 2017]. This review provides an overview of the UPR and its contribution to tumour progression from early oncogenesis to the development of chemoresistance.

**UPR signalling – The basics**

IRE1α is a type I transmembrane protein with cytosolic serine/threonine kinase and endoribonuclease (RNase) domains, which are both activated by the oligomerization of IRE1 and autophosphorylation of its kinase domain [Mori et al., 1993; Shamu & Walter, 1996; Sidrauski & Walter, 1997]. The RNase domain cleaves a 26-nucleotide intron within unspliced X Box binding Protein 1 (XBP1) or XBP1u mRNA. The resulting fragments are ligated by RNA 2′,3′-cyclic phosphate and 5′-OH ligase (RtcB) to generate spliced XBP1 or XBP1s mRNA, encoding the transcription factor XBP1s which promotes transcription of ER chaperones and components of ERAD (Figure 1) [Lee et al., 2003; Lu et al., 2014b]. Additionally, the IRE1 RNase degrades mRNAs that have a consensus cleavage site similar to that of XBP1u mRNA, through a process known as regulated IRE1 dependent decay (RIDD) (Figure 1). Targets of RIDD include microRNAs, ribosomal RNAs and ER localised RNAs encoding membrane and secreted proteins [Hollien & Weissman, 2006; Hollien et al., 2009]. IRE1 kinase activity has been less well studied but has been demonstrated to activate c-Jun N-terminal kinase (JNK) signalling by interacting with the adaptor protein TNF receptor-Associated Factor 2 (TRAF2) [Urano et al., 2000; Hu et al., 2006; Ogata et al., 2006].

PERK is also a type I transmembrane protein that is activated in a similar manner to IRE1 in response to ER stress through dissociation of GRP78 from its luminal domain. This initiates dimerization of PERK and autophosphorylation and activation of its kinase domain [Liu et al., 2000], which then phosphorylates eukaryotic translation Initiation Factor 2 subunit alpha (eIF2α) resulting in transient inhibition of mRNA translation (Figure 1) [Harding et al., 2000]. Inhibition of eIF2α allows for the selective CAP-independent expression of Activating Transcription Factor 4 (ATF4), a transcription factor that regulates adaptive genes encoding protein folding, amino acid metabolism and autophagy proteins [B’Chir et al., 2013]. In addition to its pro-survival function, ATF4 activates expression of the transcription factor CCAAT/Enhancer-Binding Protein Homologous Protein (CHOP). CHOP induction has been linked to the onset of cell death through various mechanisms including direct regulation of the cell death machinery [McCullough et al., 2001; Yamaguchi & Wang, 2004; Lu et al., 2014a] and indirectly by re-establishing protein translation, via GADD34 induction, thus intensifying ER stress and triggering cell death [Marciniak et al., 2004].

PERK activation has also been linked to the expression of anti-oxidant genes by phosphorylating the transcription factor Nuclear Factor Erythroid 2 like 2 (NRF2). This facilitates NRF2 release from an inhibitory association with Kelch-like ECH-associated
Figure 1 | Schematic representation of the UPR

Unfolded or misfolded proteins accumulate in the ER due to conditions such as hypoxia and nutrient deprivation, leading to a condition referred to as ‘ER stress’. To restore ER homoeostasis, GRP78 dissociates from each of the UPR transducers allowing for their activation. ATF6 traverses to the Golgi apparatus, where it is cleaved by site 1 proteases (S1P) and site 2 proteases (S2P) to produce an active transcription factor. Both PERK and IRE1 are activated by dimerization and autophosphorylation. PERK kinase phosphorylates eIF2α, which shuts down CAP-dependent protein translation, allowing for the select expression of ATF4. IRE1 RNase splices XBP1 mRNA to generate an mRNA encoding the active transcription factor XBP1s, while also cleaving a subset of RNAs in a process known as regulated IRE1 dependent decay (RIDD). IRE1 kinase binds TRAF2 to initiate JNK signalling. Collectively, UPR pathways coordinate to enhance protein folding, reduce the protein load by shutting down translation, and eliminate misfolded proteins via ERAD.

protein 1 (KEAP1) enabling nuclear translocation [Cullinan et al., 2003; Cullinan & Diehl, 2004]. Work by Lu et al. (2004), using an artificial construct to trigger PERK activation in the absence of ER stress, concluded all transcriptional effects downstream of PERK were dependent on eIF2α phosphorylation. How PERK-mediated NRF2 activation aligns with this observation may suggest a requirement for activation of additional arms of the UPR. Further studies are required to address the relative contributions of NRF2 and eIF2α to PERK-mediated transcriptional responses.

ATF6 is a type II transmembrane protein that is transported from the ER to the Golgi apparatus upon release by GRP78. Here ATF6 is cleaved by the Site 1 and Site 2 proteases S1P and S2P [Ye et al., 2000], releasing an active transcription factor comprising of the ATF6 cytosolic domain (Figure 1). ATF6 moves to the nucleus where it binds to ER stress response elements (ERSE), ERSE-II and UPR response elements (UPRE), promoting the transcription of XBP1 and other ER chaperones [Yoshida et al., 1998, 2001; Wang et al., 2000; Kokame et al., 2001].

The UPR in tumour initiation, development and progression

Over the past 15 years it has gradually emerged that UPR signalling contributes to tumour development. It is now well accepted that ER stress and UPR
activation contributes to the development and progression of many cancers including prostate, breast and colon cancer [Scriven et al., 2009; Sheng et al., 2015; Li et al., 2017]. Tumour progression can be broken down into various stages starting with transformation followed by unrestricted cell division, angiogenesis, invasion and metastatic spread and, significantly, the UPR has been implicated in each stage.

**Contribution of UPR signalling to cellular transformation**

Neoplastic transformation is initiated either by the loss of tumour suppressors such as p53 or by the activation of oncogenes including \( \text{BRAF}^{\text{V600E}} \), c-MYC and H-RAS. Irrespective of the initiating mechanism, cellular transformation is associated with unrestricted rapid cell division. Typically, this overwhelms the protein folding capacity of the ER, triggering UPR activation. Activation of PERK, IRE1, and to a lesser extent, ATF6-dependent signalling pathways has been reported in several cancers following oncogene activation. \( \text{BRAF}^{\text{V600E}} \) triggered IRE1 and ATF6 activation in melanoma cells [Croft et al., 2014], and c-MYC overexpressing B cells, derived from Burkitt's lymphoma patients, exhibit increased phosphorylation of PERK and eIF2\( \alpha \) and increased levels of XBP1s and ATF4 compared with B cells derived from healthy donors [Hart et al., 2012]. Selective activation of c-MYC in mouse embryonic fibroblasts (MEFs) expressing a tamoxifen inducible c-MYC (mycER) also leads to PERK and IRE1 activation. Likewise, UPR activation was reported following H-RAS activation in melanocytes and keratinocytes [Blazanin et al., 2017].

Following activation of an oncogene, initiation of UPR signalling is likely to be a key pro-survival, adaptive response employed to off-set the increased protein folding demands being placed on the ER (Figure 2). Indeed, in several models of oncogene activation, UPR activation was directly linked to increased protein misfolding, as treatment with the chemical chaperone 4-PBA reduced UPR initiation. H-RAS-activated keratinocytes that are treated with 4-PBA have reduced activation of UPR markers and cell proliferation [Blazanin et al., 2017] suggesting that, in addition to increasing ER capacity, UPR-mediated signals may also contribute in other ways to oncogene-induced hyperproliferation. The importance of this adaptive UPR for the maintenance of cell viability has been reported, with knockdown or knockout of UPR mediators being demonstrated to reduce cell survival post oncogene activation [Hart et al., 2012; Blazanin et al., 2017]. While this evidence points towards a predominantly pro-tumourigenic role for UPR signalling in tumorigenesis, it is important to note that UPR signalling has also been linked to the activation of anti-tumourigenic mechanisms such as senescence. In a model of H-RAS\(^{V12} \) activation, inhibition of IRE1 or ATF6 (via expression of dominant negative mutants) or knockdown of XBP1 or ATF4 reduced the proportion of cells entering senescence [Denoyelle et al., 2006]. A role for IRE1 and specifically IRE1-RIDD activity in cell senescence has recently been proposed. Blazanin et al. (2017) demonstrated that long-term knockdown of IRE1 reduced H-RAS induced senescence, while knockdown of XBP1 increased it, suggesting a contribution of IRE1-RIDD activity. Indeed in this model, ID1, a transcription factor linked to escape from senescence and development of cancer, was identified as an IRE1-RIDD target. This observation, while intriguing, adds another layer of complexity to the role of UPR signalling in tumorigenesis, and suggests that depending upon the model or stage of tumorigenesis, it may have opposing roles in the balance between proliferation and senescence. IRE1 mutations in glioblastoma multiforme (GBM) were recently reported by Lhomand et al. (2018), with one mutated form of IRE1 characterised by elevated RIDD activity and reduced ability to form tumours \textit{in vivo}. Whether the inability of this mutant to form tumours can be linked to enhanced senescence is unknown.

Loss of p53 expression in MEFs and various cancer cell lines has been associated with a concurrent increase in IRE1 expression and activation of the IRE1-XBP1s signalling axis. Wild type p53 was demonstrated to regulate IRE1 stability by promoting its proteasomal-mediated degradation via an IRE1 interaction with the E3 ubiquitin ligase synoviolin [Namba et al., 2015]. However, when p53 is lost, the resulting stabilisation of IRE1 increased the capacity of the ER to secrete proteins, which may allow transformed cells to sustain higher rates of proliferation. Indeed, \textit{in vivo} HCT116 tumour xenografts lacking p53 displayed increased tumour volume compared with wild-type p53 HCT116 xenografts. Treatment with an IRE1
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**Figure 2 | The role of the UPR at different stages of cancer progression**

ER stress is induced during transformation due to the increased demand for proteins to support growth. Increased protein folding capacity is enabled by a pro-survival UPR. UPR signalling supports tumour establishment by promoting cell cycle progression and cell proliferation pathways. Rapid growth decreases the availability of oxygen and nutrients in the tumour, leading to further ER stress. The UPR promotes expression of pro-angiogenic factors in response to hypoxia, resulting in angiogenesis. UPR-mediated rewiring of metabolic pathways also allows the tumour to cope with nutrient deprivation. Various cell types are recruited to the tumour to foster its development and to evade immune destruction. This results in the formation of the tumour microenvironment (TME). Once the TME is established, the UPR aids tumour cells in epithelial to mesenchymal transition (EMT) by overcoming the stress of cell detachment, and also increases EMT transcription factor expression and reduces cell–cell junction markers, promoting metastasis. During chemotherapy treatment, an adaptive UPR allows tumour cells to survive and promote drug efflux from the cell, leading to chemoresistance. UPR-induced autophagy in cancer cells and UPR-mediated expansion of cancer stem cells (CSCs) supports tumour regrowth. Abbreviations: CAF, cancer-associated fibroblast; ECM, extracellular matrix; TAM, tumour-associated macrophage; Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell.

RNase inhibitor STF-083010 reduced growth of both HCT116p53<sup>−/−</sup> and p53<sup>+/+</sup> xenografts. However, the suppression in tumour growth was more pronounced in HCT116p53<sup>−/−</sup> compared with HCT116p53<sup>+/+</sup> xenografts, suggesting there may be a therapeutic benefit to targeting IRE1 in p53 null cancers.

Although activation of UPR signalling helps cells meet the protein folding demands instigated during transformation, it must be carefully controlled to avoid initiation of cell death. This presents cancer cells with a conundrum – how to harness the beneficial effects of UPR activation to sustain cell division while avoiding initiation of cell death signals.

Too little UPR signalling will be unable to support sustained proliferation, while too much will initiate cell death. Cells must find the elusive "sweet spot" where the amplitude of UPR activation is adequate to sustain cell division, but not extreme enough to lead to cell death. One mechanism by which cells can maintain UPR activation while avoiding initiation of cell death is to actively suppress components of the cell death pathway or stimulate cytoprotective mechanisms such as autophagy. In RAS-transformed MEFs, CHOP mRNA stability was down-regulated by oncogenic H-RAS and addition of exogenous CHOP in H-RAS expressing cells prevented the formation of foci, a marker of cellular transformation.
[Rong et al., 2005]. Following c-MYC activation, PERK signalling was demonstrated to suppress cell death through the activation of autophagy [Hart et al., 2012]. Exactly how autophagy attenuates cell death post c-MYC activation is not known. However, it has been proposed that elevated autophagy may be cytoprotective by removing damaged/unfolded proteins, thereby reducing proteotoxic stress in c-MYC expressing cells. The increase in PERK-dependent autophagy was specifically linked to induction of ER stress, as treatment of cells with 4-PBA blocked degradation of the autophagy marker p62. Ablation of PERK expression was also associated with increased occurrence of cell death markers in vitro and reduced tumour formation in vivo [Hart et al., 2012].

UPR-mediated killing may eliminate cells expressing high oncogene activation and select those cells with either lower oncogene activation or defective death pathways. In a H-RAS\textsuperscript{V12} driven tumorigenesis model, long-term suppression of IRE1, ATF6, XBP1 or ATF4 induced cell death, but also lead to the selection of cells expressing lower levels of oncogene activation and defective XBP1s and ATF4 signalling pathways [Denoyelle et al., 2006].

While internal stresses such as oncogene activation or loss of tumour suppressors undoubtedly trigger ER stress, it is also important to consider the environment in which cancer cells exist and incorporate this into cellular models. Most studies examining UPR signalling during cellular transformation have focused solely on the outcome of internal stresses triggered by oncogene activation or loss of a tumour suppressor. However, we know that cancer cells, especially in the very early stages of tumorigenesis, exist within a harsh environment characterised by acidosis, limited glucose availability and hypoxia. Exposure of cancer cells to this environment initiates alterations in metabolic programs to support anabolic growth. Tumour cells become reliant on glucose to fuel hyperproliferation leading to a low glucose environment, which triggers further UPR signalling. Huber et al. (2013) recently demonstrated, using an inducible model of RET oncogenic signalling in Rat-1 cells, that glucose deprivation initiated ER stress and ER stress-induced cell death, which was associated with elevated CHOP expression. Deletion of CHOP attenuated ER stress-induced cell death, indicating an anti-tumourigenic role for PERK signalling under a combination of oncogene activation and environmental conditions representative of the tumour microenvironment. While this metabolic crisis did initiate death in a large proportion of cells, surviving cells continued to proliferate leading to tumour formation. Analysis of the surviving population found these cells had adapted by suppressing PERK-CHOP signalling through increased expression of p58\textsuperscript{IPK} [Huber et al., 2013]. Originally described as an inhibitor of both PKR [Lee et al., 1994] and PERK [Yan et al., 2002; Van Huizen et al., 2003], p58\textsuperscript{IPK} has also been demonstrated to promote GRP78 activity [Rutkowski et al., 2007] and co-translocational ER protein degradation [Oyadomari et al., 2006]. Examination of downstream signalling in cells following metabolic crisis found these cells had adapted by increasing p58\textsuperscript{IPK} expression and suppressing PERK-CHOP signalling, although the precise mechanism was unclear [Huber et al., 2013]. This study again illustrates the importance of the amplitude of UPR signalling in cells during oncogenesis. Those cells that overcome oncogenic stresses during early cancer progression gain cytoprotective mechanisms, such as P58\textsuperscript{IPK} activation, and resist cell death from further stresses, shifting the balance function of the UPR from pro-apoptotic to pro-survival.

In addition to these classical roles for ER stress/UPR activation in cancer, recent studies have raised the possibility that mutations within UPR mediators themselves could have the potential to drive the development of cancer. Patient genome sequencing analysis from various cancer types revealed ERN1, the gene encoding IRE1, was in the top 20 mutated protein kinases carrying at least one driver mutation [Greenman et al., 2007]. Chevet et al. (2016) have reviewed data documented in the Catalogue of Somatic Mutations in Cancer, cBIOportal and IntOgens databases, which illustrates that each of the UPR transducers is enriched in mutations in different types of cancer [Forbes et al., 2009; Cerami et al., 2012; Gao et al., 2013; Gonzalez-Perez et al., 2013]. As yet, the functional significance of mutations in UPR mediators has not been explored. It would be interesting to assess the impact of these mutations on downstream UPR signalling. Can such mutations modulate or rewire UPR networks in such a way as to benefit cells undergoing transformation?

In summary, to successfully navigate the process of transformation, cancer cells must find ways to adapt to the significant internal stresses associated
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with oncogene activation or loss of tumour suppressors. As outlined, activation of the UPR represents a process that cancer cells can utilise to safeguard their continued survival. However, UPR activation must be carefully controlled to ensure it stays within the adaptive, pro-survival phase or else cell death ensues. In this way, transformation can be thought of as a bottleneck which positively selects for those cancer cells that have either the right level of ER stress that can be counterbalanced by an adaptive UPR, or cells which have rewired their UPR signalling network to actively suppress terminal pro-death signalling.

UPR regulation of tumour growth

Following successful transformation, the next challenge solid cancers face is to ensure their continued growth and spread to other sites in the body. The IRE1-XBP1s pathway has been linked to cell proliferation in several cancers including colon cancer, breast cancer, prostate cancer and melanoma. Knockdown of IRE1 expression in a panel of colon cancer cell lines reduced cell proliferation in vitro and in vivo [Li et al., 2017]. Similarly, inhibiting IRE1 RNase activity reduced the proliferation of breast cancer cells in vitro [Logue et al., 2018]. The observed reduction in proliferation was not associated with increased cell death but was linked in vitro to the arrest of cells in G1 [Li et al., 2017, Logue et al., 2018], indicating IRE1 signalling can impact on cell cycle dynamics. The Cyclin D1:CDK4 complex promotes movement through the G1 phase of the cell cycle by inhibiting retinoblastoma protein. Knockdown of IRE1 in colon cancer cells led to the activation of PERK, which resulted in a block in protein translation due to eIF2α phosphorylation. The resulting reduction in β-catenin translation decreased cyclin D1 expression [Li et al., 2017], providing rationale for the reduction in cell proliferation. Likewise in prostate cancer, IRE1 expression has been linked to the maintenance of another member of the cyclin family cyclin A1, reinforcing the importance of IRE1 signalling in maintaining cell division in cancer cells [Thorpe & Schwarze, 2010]. While the IRE1 axis has been demonstrated to promote cell proliferation, the relationship between the UPR and cell cycle control is complex. PERK activation, while being linked to G1 arrest via cyclin D1 depletion, has also been associated with suppression of the G2 phase of the cell cycle [Malzer et al., 2010] and inhibition of DNA synthesis [Cabrera et al., 2017]. How these opposing outcomes balance within a tumour setting is not known, but it is important that we appreciate the complexity of these pathways when considering therapeutic rationales.

Surviving hypoxia and nutrient deprivation through UPR activation

In addition to maintaining high proliferative capacity, cancer cells, especially in the early stages of tumour establishment, are faced with an additional challenge – sustaining proliferation and viability in a hypoxic and nutrient deprived environment. Tumours rapidly outgrow their available blood supply resulting in heterogeneous oxygen availability throughout the tumour, with some regions having little or no oxygen. Levels of ROS increase within cells exposed to hypoxia, heightening ER stress and UPR activation [Harding et al., 2003; Bobrovnikova-Marjon et al., 2010]. Activation of both the IRE1 and PERK arms of the UPR aids cell survival in hypoxic conditions (Figure 2). Cells devoid of XBP1 display reduced survival in vitro following exposure to hypoxic conditions [Romero-Ramirez et al., 2004]. Moreover, transformed XBP1 null MEFs display reduced xenograft formation in vivo compared with their wild-type counterparts [Romero-Ramirez et al., 2004], underscoring the importance of XBP1s signalling in adapting to hypoxic conditions. Precisely how the IRE1-XBP1s signalling axis helps sustain cell survival during hypoxia is not fully understood, but a recent report suggests it may be through interaction with HIF1α [Chen et al., 2014].

Stabilisation of the HIF family of transcription factors represents one of the most important pathways of cellular adaptation to hypoxic conditions. Under normoxic conditions, HIF1α is hydroxylated to enable its ubiquitination by VHL E3 ubiquitin ligase and subsequent removal via proteasomal degradation [Majmundar et al., 2010]. Following exposure to hypoxic conditions, HIF1α hydroxylation is prevented, allowing for protein stabilisation and activation of downstream signalling. HIF1α has a diverse range of downstream target genes that are implicated in growth and survival, glucose metabolism and angiogenesis [Majmundar et al., 2010]. Activation of HIF1α-dependent signalling is a key event in dictating cellular adaptation and survival under hypoxia. In a model of triple negative breast cancer
(TNBC), Chen et al. (2014) demonstrated that HIF1α is dependent on XBP1s for efficient transcription of HIF1α downstream target genes. The authors demonstrated that XBP1s–HIF1α complex formation on the promoters of target genes was crucial for their transcription, via the recruitment of RNA polymerase II. Based on these observations, it seems that the IRE1-XBP1s axis, at least in TNBC, helps to sustain the HIF1α transcriptional program; thereby promoting adaptive responses and aiding tumour survival.

In addition to IRE1 signalling, co-option of the PERK pathway has also been implicated in cell survival under hypoxic conditions [Bi et al., 2005]. Cells devoid of PERK expression or cells expressing a non-phosphorylatable version of eIF2α exhibit substantially reduced cell survival in vitro following exposure to hypoxia [Bobrovnikova-Marjon et al., 2010]. Likewise, in vivo xenografts established from PERK−/− cells displayed reduced tumour volume compared with xenografts derived from PERK competent cells, underscoring the importance of a functional PERK pathway in the adaptation to hypoxic conditions. Activation of PERK-dependent signalling, through increased expression of ATF4 and CHOP, helps off-set the negative consequences of increased ROS production through increasing antioxidant responses [Harding et al., 2003; Marciniak et al., 2004]. Aside from aiding cell survival by counteracting hypoxia-induced oxidative stress, PERK-mediated signalling has also been demonstrated to sustain cell viability by triggering autophagy.

Autophagy is a vital cellular process, which enables the degradation of proteins, cytoplasmic content and damaged organelles. Exposure to cellular stresses such as hypoxia and nutrient deprivation has been reported to increase autophagy levels [Glick et al., 2010]. This benefits the cell by removing damaged proteins and providing a source of recycled nutrients to sustain proliferation and survival. Elevated autophagy has been observed both in vitro in cancer cells lines in response to hypoxia or nutrient deprivation, and in vivo tumour xenografts, where increased autophagy levels correlated with hypoxic regions of the tumour [Rouschop et al., 2010]. Increased transcription of key autophagy related genes, ATG5 and MAP1LC3B, has been linked to PERK-dependent up-regulation of the transcription factors ATF4 and CHOP [Rouschop et al., 2010].

Inhibition of the PERK-eIF2α-ATF4 signalling axis reduced autophagy induction, thereby sensitising cells to hypoxia [Rouschop et al., 2010].

**UPR regulation of tumour metabolism**

In addition to adapting to hypoxic conditions, cancer cells must also contend with reduced nutrient supply until sufficient tumour vascularisation occurs. To counteract these low nutrient conditions, cancer cells utilise several approaches including UPR-dependent rewiring of their metabolic pathways (Figure 2). The hexosamine biosynthetic pathway (HBP) generates uridine diphosphate N-acetylgalactosamine (O-GlcNAc), which serves as a substrate for O-GlcNAc transferase (OGT) by facilitating transfer of the GlcNAc moiety onto the free hydroxyl of select serine and threonine residues of a target protein [Hanover et al., 2010]. Up-regulation of the HBP has been reported in several cancers [Slawson & Hart, 2011]. Recently, breast cancer maintenance of OGT levels has been demonstrated to sustain expression of HIF1α, such that knockdown of OGT reduced HIF1α expression leading to glycolytic defects [Ferrer et al., 2014]. Therefore, elevating the HBP aids metabolic reprogramming of cancer cells. Recently the UPR has been identified as an upstream activator of the HBP [Wang et al., 2014; Chaveroux et al., 2016]. Overexpression of XBP1 or induction of a UPR response, by either physiological stresses or treatment with pharmacological drugs, elevated the levels of key HBP-related enzymes [Wang et al., 2014]. Conversely, knockdown of XBP1 blocked starvation-induced increases in O-GlcNAc modification [Wang et al., 2014]. Upon glucose deprivation, ATF4 has been demonstrated to promote the expression of glutamine:fructose-6-phosphate aminotransferase 1 (GFAT1), the first rate-limiting enzyme in the HBP and driver of HBP flux [Chaveroux et al., 2016]. Knockdown of ATF4 resulted in reduction in Gfat1 mRNA and prevented glucose deprivation-induced O-GlcNACylation [Chaveroux et al., 2016]. Collectively, these data suggest that induction of the UPR regulates O-GlcNAc modification and aids metabolic reprogramming of cancer cells. At present, relatively few studies have focused on dissecting the reliance of cancer cells on UPR-mediated control of the HBP. Further studies are required to understand the impact of UPR-HBP crosstalk in altering cellular metabolism in cancer.
UPR-mediated suppression of anti-tumour immune responses

While finding ways to circumvent the challenges associated with limited oxygen and nutrient supply tumour cells must also mitigate the anti-tumour immune response to ensure continued growth. Cancer cells can evade immune surveillance by producing immunosuppressive factors that both diminish the anti-tumour immune response [Balkwill et al., 2005] and re-educate the infiltrating immune cells [Pollard, 2004] (Figure 2). Emerging evidence suggests UPR signalling may contribute to the active suppression or modulation of immune responses in several ways. ER stressed cancer cells have been reported to secrete, as yet unidentified, soluble factors, which transmit ER stress to immune cells in a process known as Transmissible ER Stress (TERS). TERS-exposed myeloid dendritic cells (DCs) display an activated state associated with production of pro-tumorigenic cytokines and increased levels of the T cell suppressive enzyme arginase [Mahadevan et al., 2012]. In vitro, TERS-primed DCs displayed an impaired ability to cross present antigen and cross prime CD8+ T cells [Mahadevan et al., 2012]. While in vivo, combination of TERS-primed DCs with tumour cells in naïve immunocompetent mice resulted in accelerated tumour growth, associated with decreased infiltration CD8+ T cells. Similar results have also been reported in macrophages where incubation with conditioned medium from ER stressed cancer cells increased expression of ER stress response genes and pro-inflammatory cytokines [Mahadevan et al., 2011; Cullen et al., 2013]. These data suggest that ER stress-mediated factors, released from cancer cells, can modulate the immune response to promote tumour progression. Further studies are required to reveal the identity of these transmissible factors and to understand how this facilitates alterations in immune cell function.

In addition to regulating the function of immune cells by transmitting ER stress, UPR signalling has also been reported to decrease expression of cell surface ligands on cancer cells including MHC Class I and the NK cell activating receptor ligands MICA/B [de Almeida et al., 2007; Granados et al., 2009; Fang et al., 2014]. While this suggests UPR activation could promote tumour development by suppressing Natural Killer and CD8+ T cell mediated cytotoxicity further studies, within the setting of cancer, are required to address this question.

UPR and angiogenesis

Tumours can overcome the limitations and stresses that result from an oxygen- and nutrient-deprived environment partly by stimulating vascularisation through the formation of new blood vessels (angiogenesis). Without angiogenesis, tumour growth is stunted and tumours are unable to grow beyond 1–2mm³. Angiogenesis occurs through the sprouting, migration and proliferation of endothelial cells, and is regulated via the secretion of soluble factors including vascular endothelial growth factor-A (VEGF-A), fibroblast growth factor (FGF) and platelet-derived growth factor with VEGF-A being one of the most characterised pro-angiogenic factors [Yancopoulos et al., 2000]. HIF1α stabilisation is a well-studied mechanism that facilitates VEGF-A production under hypoxia. In TNBC, XBPIs expression was required to facilitate efficient HIF1α-mediated VEGF-A production and angiogenesis [Chen et al., 2014]. XBPIshRNA expressing cells produced less VEGF-A transcript following induction of hypoxic conditions, and in vivo xenografts derived from these cells displayed reduced angiogenesis compared with their wild-type counterparts [Chen et al., 2014]. Exposure to ER stress, induced by either pharmacological drugs or physiological conditions such as glucose deprivation, has also been demonstrated to induce VEGF-A production in a HIF1α-independent manner (Figure 2.) [Ghosh et al., 2010; Wang et al., 2012]. IRE1−/− MEFS displayed reduced VEGF-A production following initiation of ER stress by a range of physiological and pharmacological inducers [Ghosh et al., 2010]. Expression of an IRE1 dominant negative construct in A549/8 cells and U87 cells also reduced glucose deprivation-mediated increases in VEGF-A [Drogat et al., 2007]. In vivo, IRE1 dominant negative U87 tumour xenografts were smaller in size and were characterised by reduced blood vessel formation, further underscoring a reliance of these tumours on the IRE1 signalling axis for efficient angiogenesis. In addition to VEGF-A, inhibition of the IRE1 pathway has been linked to decreased expression of other pro-angiogenic mediators, including IL-6 and IL-8 [Auf et al., 2010]. Taken together these observations suggest IRE1 signalling is predominantly
pro-angiogenic. However, recent work characterising IRE1 somatic variants in human GBM found that mutations resulting in increased IRE1-XBP1s signalling stimulated angiogenesis, while mutations elevating IRE1-RIDD activity suppressed angiogenesis [Lhomond et al., 2018]. This observation suggests that the balance between IRE1-XBP1s and IRE1-RIDD signalling could influence whether a pro-angiogenic or anti-angiogenic outcome is favoured.

In addition to the IRE1 signalling axis, PERK-mediated processes have also been linked to production of pro-angiogenic factors. Knockdown of PERK reduced glucose deprivation-driven increases in VEGF-A, IL6 and FGF transcripts in SCC-81B cells [Wang et al., 2012], and PERK knockout MEFs displayed reduced VEGF-A production in response to treatment with thapsigargin [Ghosh et al., 2010]. In vivo xenografts established from either PERK knock-out or knockdown cells display reduced growth, with the resulting tumours displaying reduced blood vessel formation compared with PERK competent controls [Blais et al., 2006; Wang et al., 2012]. Subsequent studies have found that PERK mediated control of angiogenic factors is dependent on ATF4, and ATF4 has also been demonstrated to bind to the VEGF-A promoter [Ghosh et al., 2010; Wang et al., 2012]. While most data linking UPR signalling to production of pro-angiogenic factors implicates IRE1- and PERK-dependent mechanisms, ATF6 may also contribute as knockdown of ATF6 has been reported to reduce thapsigargin-induced up-regulation of VEGF-A transcript in HepG2 cells [Ghosh et al., 2010]. This may be an indirect consequence of ATF6 signalling, through the regulation of XBP1 mRNA feeding into the IRE1 pathway. However, overexpression of cleaved ATF6 in 293T cells expressing a VEGF-A promoter reporter construct suggested a direct interaction may occur [Ghosh et al., 2010].

**Invasion and metastasis**

Angiogenesis provides an avenue for tumour cells to escape and metastasise to other organs but before gaining the ability to enter the vascular circulation, tumour cells must acquire an invasive, migratory phenotype. Epithelial to mesenchymal transition (EMT) is an essential cellular process during embryonic development, playing an important role in mesoderm formation and the migration of neural crest cells [Thiery et al., 2009]. However, in cancer, abnormal activation of EMT causes tumour cells to lose cell to cell contacts, thus conferring an invasive, migratory phenotype that favours metastasis. The process of EMT is driven by several transcription factors, including Snail1, Snail2, Zeb1, Zeb2 and Twist1 [Puisieux et al., 2014]. Activation of these transcription factors drives EMT by down-regulating cell adhesion markers such as E-cadherin and up-regulating mesenchymal markers such as vimentin, resulting in the acquisition of a migratory phenotype [Puisieux et al., 2014].

Induction of ER stress has been reported to induce EMT in vitro and in vivo [Lawson et al., 2008; Tanjore et al., 2011; Zhong et al., 2011; Paller, 2012], with both the IRE1-XBP1s and PERK-eIF2α-ATF4 signalling pathways being implicated [Tanjore et al., 2011; Feng et al., 2014; Cuevas et al., 2017]. In the rat alveolar epithelial cell line RLE6TN, treatment with thapsigargin was reported to trigger EMT in a SMAD2/3 and Src-dependent manner [Tanjore et al., 2011]. Knockdown of IRE1 prevented activation of SMAD2/3 and Src-dependent pathways; however, the levels of EMT markers were not examined after IRE1 knockdown in this model [Tanjore et al., 2011]. XBP1 knockdown in the mesenchymal TNBC cell line MDA-MB-231 reverts their phenotype to a more epithelial phenotype, characterised by increased expression of E-cadherin and a restoration of cell to cell junction formation [Li et al., 2015]. IRE1-XBP1s signalling has also been implicated in EMT induced by the lysyl oxidase family member, lysyl oxidase-like 2 (LOXL2) [Peinado et al., 2005; Cuevas et al., 2017]. Activation of IRE1 was dependent on the sequestering of GRP78 by LOXL2, which resulted in XBP1s-dependent up-regulation of Snail1, Snail2, Zeb2 and Tcf3 transcription factors. Inhibition of IRE1 RNase activity, by the small molecule inhibitor 4μ8c, reduced LOXL2-mediated loss of E-cadherin and blocked transition to a spindle morphology that is typical of cells post-EMT [Cuevas et al., 2017]. These studies indicate that loss of IRE1 signalling should reduce the invasive and migratory capacity of cells by suppressing EMT. However, knockdown of IRE1 in glioma cell lines has been associated with increased invasion. Stable expression of an IRE1 dominant negative construct in U87 cells increased cell migration, which was linked to enhanced expression of the extracellular matrix.
(ECM) protein and RIDD target SPARC [Dejeans et al., 2012]. Recent work stratifying GBM patients into groups of high (+) or low (−) XBP1s activity in combination with high or low RIDD activity reached a similar conclusion, where XBP1+/−/RIDD−/− tumours associated with a more mesenchymal phenotype in comparison with XBP1+/−/RIDD+ tumours [Lhomond et al., 2018]. Again, these observations highlight the importance of understanding the finer nuances of IRE1 RNase activity and how its targeting of XBP1 versus RIDD can favour different outcomes.

The PERK-eIF2α arm of the UPR has also been linked to acquisition of a mesenchymal phenotype. Increased phosphorylation of PERK and eIF2α, and elevated expression of GADD34 has been reported in cells that have undergone EMT, and inhibition of constitutive PERK activity reduced the ability of cells to migrate in vitro [Feng et al., 2014]. Likewise, pre-treatment of 4T1 cells with a PERK inhibitor diminished their metastatic capacity in vivo [Feng et al., 2014]. Analysis of gene expression microarray data from 792 breast, colon and gastric tumours reported a strong association between EMT markers and ATF4 [Feng et al., 2014]. These data suggest that targeting the PERK pathway may decrease the invasive and migratory capacity of cells that underwent EMT.

Once tumour cells enter the blood stream, the next challenge they face is to maintain their survival under anchorage independent conditions. Detachment from the ECM has been reported to trigger a drop in ATP levels, leading to a rapid increase in the production of ROS [Schafer et al., 2009]. To off-set the stresses of ROS, PERK signalling is activated in cells that have undergone EMT, and inhibition of constitutive PERK activity reduced the ability of cells to migrate in vitro [Feng et al., 2014]. Analysis of gene expression microarray data from 792 breast, colon and gastric tumours reported a strong association between EMT markers and ATF4 [Feng et al., 2014]. These data suggest that targeting the PERK pathway may decrease the invasive and migratory capacity of cells that underwent EMT.

UPR and chemoresistance

Patients undergoing chemotherapy initially respond to treatment, as evident by regression of the tumour. However, many develop resistance to chemotherapy due to a small population of cancer cells that adapt intrinsically to cope with this additional stress. Adaptation to chemotherapeutics can occur due to factors such as enhanced drug efflux, drug activation/inactivation and alterations in drug targets that promote elimination of the drug from the target [Holohan et al., 2013]. Tumour heterogeneity also presents a challenge due to a phenotypically diverse population of cells that may respond differently to the same treatment [Dagogo-Jack & Shaw, 2018]. For example, chemotherapies tend to target the rapidly dividing cell population, but have little effect on the relatively quiescent cancer stem cell (CSC) population. CSCs tend to be inherently resistant to chemotherapy due to heightened expression of metabolizing enzymes such as aldehyde dehydrogenase, anti-apoptotic BCL2 family members [Abdullah & Chow, 2013; Holohan et al., 2013] and hypoxia-inducible factors [Generali et al., 2006].

Harnessing the adaptive power of the UPR is one mechanism exploited by cancer cells to ensure survival upon exposure to chemotherapeutic agents, with all three arms of the UPR being implicated (Figure 2). Knockdown of GRP78, ATF6, ATF4 and XBP1s has been linked to the resensitisation of chemoresistant cancer cells [Wang et al., 2009; Kim et al., 2016; Chen et al., 2017]. XBP1s has been implicated in promoting relapse of TNBC tumours in vivo.
Following treatment with doxorubicin, MDA-MB-231 xenografts display an initial reduction in tumour volume followed by tumour regrowth after therapy withdrawal [Chen et al., 2014]. Stable knockdown of XBP1 in MDA-MB-231 tumour xenografts prevented tumour regrowth following doxorubicin withdrawal. Moreover, inhibition of IRE1 RNase activity via the small molecule inhibitor MKC8866 also prevented the regrowth of MDA-MB-231 xenografts after paclitaxel withdrawal [Logue et al., 2018], suggesting a dependency on XBP1s signalling for tumour regrowth. Examination of TNBC patient data sets supports such a relationship, with elevated expression of an XBP1-dependent gene signature positively correlating with poor relapse free survival [Chen et al., 2014]. Therapy-driven expansion of CSCs represents one mechanism by which tumour relapse can be instigated. CSCs are characterised by their ability to form tumour spheres/mammospheres and have a characteristic CD44<sup>high</sup>/CD24<sup>low</sup> staining pattern. Knockdown of XBP1 decreased the proportion of CD44<sup>high</sup>/CD24<sup>low</sup> cells post chemotherapy and reduced the ability of these cells to form mammospheres in vitro [Chen et al., 2014]. Likewise, inhibiting IRE1 RNase activity reduced the ability of MDA-MB-231 cells to form mammospheres post-paclitaxel treatment, which was attributed to IRE1-mediated reduction in pro-tumourigenic factors [Logue et al., 2018].

In addition to the IRE1-XBP1s axis, PERK dependent signalling processes have also been linked to CSC expansion. Cervical CSCs are resistant to ER stress-induced apoptosis and only display PERK activation [Fujimoto et al., 2016]. This effect seems to be PERK specific, as pharmacological inhibition of PERK and not IRE1 sensitises CSCs to ER-stress induced apoptosis. However, when these CSCs are treated with cisplatin, they switch from a reliance on PERK to IRE1, which is demonstrated when a combination of cisplatin and 4μ8C induces apoptosis in CSCs. This could suggest that CSCs overcome stresses inflicted during tumour progression by activating PERK. However, exposure to the additional stress of chemotherapy forces the CSCs to switch from PERK to IRE1 to potentially avoid CHOP-induced cell death.

In contrast, Salaroglio et al. (2017) show that dual ER stress-chemotherapy resistant colon cancer cells have enhanced surface expression of the ABC transporter MDR-related protein 1 (MRP1) and activation of the PERK/NRF2 arm of the UPR. ChIP analysis also shows increased NRF2 bound to the ABBC1/MRP1 promoter, which is virtually absent in ER stress-chemotherapy sensitive cells. This observation leads to the possibility that CSCs and proliferating tumour cells may use different axes of the UPR to complement their own established oncogenic mechanisms to overcome chemotherapy. Additionally, selective activation of one axis of the UPR may be dependent on other intrinsic mechanisms that are driving chemoresistance.

Conclusions and future perspectives
Classically, the UPR is considered a pro-survival adaptive mechanism aimed at reducing levels of unfolded proteins and restoring ER homoeostasis. While this may be true, especially in the early stages of tumorigenesis, cancer cells have clearly co-opted the UPR in diverse ways to benefit their progression and dissemination. As detailed in this review article, the true impact of UPR activation is far-reaching and extends well beyond maintaining ER homoeostasis. What was thought of as simply an adaptive pro-survival pathway is now emerging as a viable novel therapeutic target for the treatment of many cancers. The recent development of small molecule inhibitors targeting selective arms of the UPR suggests therapeutically targeting this pathway is within reach. Most focus has been placed on targeting the IRE1 pathway in cancer cells with recent studies using the small molecule IRE1 inhibitor (MKC8866) demonstrating tumour suppressive outcomes in pre-clinical models of triple negative breast cancer [Logue et al., 2018; Zhao et al., 2018]. For a comprehensive overview of UPR inhibitors and their clinical potential please see the recent article by Almanza et al. (2018). While the influence of UPR signalling on many facets of cancer progression has been clearly demonstrated, we currently lack an understanding of the finer subtleties governing UPR signalling in cancer cells. This is clearly illustrated by IRE1, where the balance between IRE1-XBP1s and IRE-RIDD can lead to very differing outcomes. What controls the shift from IRE1-XBP1s to IRE1-RIDD and vice versa is not understood.

Over the past decade, we have made significant advances in understanding how UPR signalling can be...
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co-opted by cancer cells. The challenge for researchers in this field is now to understand the intricacies of UPR signalling in cancer, and, using this knowledge, to develop specific and selective tools to identify those patients that are most likely to benefit from therapeutic intervention.

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