Induction of endoplasmic reticulum stress as a strategy for melanoma therapy: is there a future?

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Practice points

- Melanomagenesis is associated with genetic mutations that confer resistance to endoplasmic reticulum (ER) stress-induced apoptosis mediated by the activation of the adaptive unfolded protein response (UPR).
- Oncogenic *NRAS* and *BRAF* induce cytoprotective UPR signaling in order to actively inhibit ER stress-induced apoptosis and promote melanoma progression.
- Melanoma cells become dependent upon increased chaperone protein expression and adaptive UPR signaling for continued survival.
- Increased BCL-2 expression reduces the steady-state ER Ca^{2+} concentration in order to inhibit ER stress-induced apoptosis of melanoma.
- Inhibition of PERK or IRE1 α in order to block cytoprotective UPR signaling may sensitize melanoma cells to ER stress-induced apoptosis.
- Targeting ER chaperone proteins, such as Grp78/BiP, HSP90 and/or protein disulfide isomerase, in order to inhibit protein folding and quality control mechanisms may induce or sensitize melanoma cells to ER stress-induced apoptosis.
- Induction of ER stress in order to overload adaptive UPR signaling and induce apoptosis may provide a potential therapeutic approach for melanoma.

SUMMARY Melanoma cells employ several survival strategies, including induction of the unfolded protein response, which mediates resistance to endoplasmic reticulum (ER) stress-induced apoptosis. Activation of oncogenes specifically suppresses ER stress-induced apoptosis, while upregulation of ER chaperone proteins and antiapoptotic BCL-2 family members increases the protein folding capacity of the cell and the threshold for the induction of ER stress-induced apoptosis, respectively. Modulation of unfolded protein response signaling, inhibition of the protein folding machinery and/or active induction of ER stress may thus represent potential strategies for the therapeutic management of melanoma. To this aim, the present article focuses on the current understanding of how melanoma cells avoid or overcome ER stress-induced apoptosis, as well as therapeutic strategies through which to harness ER stress for therapeutic benefit.

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Targeted melanoma therapy with new drugs holds great promise. For example, selective MAPK pathway inhibitors show unprecedented response rates, although onset of resistance is common. In addition, targeted immunotherapies, such as monoclonal antibodies against the T-cell membrane proteins CTLA-4 and PD-1 or the ligand PD-L1, show encouraging results [1]. However, we still cannot claim that there is a cure for metastatic melanoma. Therefore, novel biomarkers of disease progression and effective treatment strategies for metastatic disease are urgently required. Melanoma development is associated with genetic alterations such as oncogenic mutations in the MAPK pathway, suppression of APAF-1, amplification of cyclin-D1 and/or loss of PTEN, which activate survival signaling in order to increase invasion and proliferation while suppressing apoptosis. However, progression of melanoma is probably driven by secondary events, such as the induction of endoplasmic reticulum (ER) stress, which is accompanied by constitutive activation of the adaptive unfolded protein response (UPR), conferring resistance to ER stress-induced apoptosis. Therefore, the dependence of melanoma cells upon UPR signaling for continued survival, which is not a requirement of normal cells, may be exploited for therapeutic benefit by targeting the induction of ER stress in order to drive apoptosis of melanoma cells.

ER stress & the UPR

The ER is a membrane-enclosed organelle with a neutral pH, high Ca^{2+} concentration (5 mM within the ER compared with 0.1 μM in the cytosol) and a low reduced:oxidised glutathione ratio (3:1 within the ER compared with 100:1 in the cytosol) [2]. The ER contains a number of specialist chaperone proteins, including Ca^{2+} -dependent calreticulin, PDI and Grp78/BiP (Figure 1), which aid correct protein folding and mediate various post-translational modifications (e.g., N-linked glycosylation, disulfide bond formation, lipidation, hydroxylation and oligomerization, among others) [3], collectively promoting cellular homeostasis. However, if a cell is exposed to hostile conditions, such as increasing temperature, hypoxia, glucose starvation or perturbed calcium balance, normal homeostasis within the ER is disrupted, resulting in increased protein misfolding [4]. In addition, viral infection or genetic/epigenetic mutations can also increase the rate of protein misfolding due to the increase

in translation, which places a larger burden on the ER [5,6]. The accumulation of damaged and misfolded proteins within the ER lumen and the imbalance of ER homeostasis are collectively referred to as 'ER stress' (Figure 1). If ER stress continues unabated, then misfolded and damaged proteins aggregate within the cell in order to form inclusion bodies that eventually result in necrotic cell death [7]. In order to counteract such events, three transmembrane receptors on the ER – PERK, IRE1 α and ATF6 – monitor the levels of misfolded proteins within the ER lumen through reversible binding to the chaperone protein Grp78/BiP [8]. During normal conditions, this interaction inhibits the activity of PERK, IRE1 α and ATF6, but in response to ER stress, these receptors are released as Grp78/BiP, which preferentially binds to misfolded proteins. PERK, IRE1 α and ATF6 subsequently activate a series of adaptive response mechanisms, collectively referred to as the UPR [9]. However, if this adaptive response is unable to reverse the damage that has been caused, or if the level of ER stress is excessive or persistent, UPR signaling will result in irreversible senescence or the activation of apoptosis (Figure 1) [10].

ER signaling in melanoma

While the UPR is primarily a cell survival mechanism that allows adaptation to adverse environmental conditions, the switch from prosurvival to proapoptotic UPR signaling also acts as a vital tumor-suppressor mechanism [11]. Therefore, melanoma cells, which are often subjected to ER stress due to transformation-dependent metabolic demands and hostile environmental factors such as increased hypoxia, nutritional stress or pH stress [12], must acquire mutations that avoid or overcome ER stress-induced apoptosis. During the initial stages of melanoma development, ER stress-induced senescence and apoptosis may even drive melanoma progression by selecting for those cancer cells that are most adapted to cope with ER stress. For example, $\text{HRAS}^{\text{G12V}}$ expression in melanocytes induces rapid cell cycle arrest, vacuolization and expansion of the ER, driven by UPR signaling through CHOP/Gadd153, Grp78/BiP and ATF4, as well as by activation of PI3K/AKT signaling [13]. However, expression of mutant NRAS or BRAF induces senescence of melanocytes without enlargement of the ER or induction of UPR signaling. Therefore, the bias against *HRAS* mutations in human melanoma may be

Figure 1. Factors mediating endoplasmic reticulum stress and the unfolded protein response. The chaperone protein Grp78/BiP within the ER aids protein folding, maintaining the three transmembrane receptors – PERK, IRE1α and ATF6 – in an inactive state. Induction of ER stress results in the accumulation of unfolded proteins within the ER lumen that preferentially bind Grp78/BiP, causing the release and activation of PERK, IRE1α and ATF6, accompanied by Ca²⁺ release from IP3R (white boxes). Adaptive UPR signaling (green boxes) counteracts the effects of ER stress in order to maintain normal cellular homeostasis. Excessive or persistent ER stress, however, results in a switch to cytotoxic UPR signaling (red boxes), resulting in apoptosis.

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ER: Endoplasmic reticulum; ERAD: Endoplasmic reticulum-associated degradation. MOMP: Mitochondrial outer membrane permeabilization; PC: Phosphatidylcholine; RIDD: Regulated IRE1-dependent decay; UPR: Unfolded protein response.

due to the induction of an ER stress response, while mutations of NRAS or BRAF may allow tumor progression due to the lack of ER stress-induced senescence or apoptosis. Furthermore, Denoyelle *et al.* also provide data indicating that mutant NRAS and BRAF may induce cytoprotective UPR signaling in order to actively inhibit ER stress-induced apoptosis and promote tumor progression [13], which is further supported by recent research demonstrating that inhibition of BRAF^{V600E} by the selective inhibitor vemurafenib results in ER stress-induced apoptosis of BRAF^{V600E}-mutated melanoma cells [14].

While IRE1α and ATF6 signaling resulting from ER stress is rapidly attenuated in normal cells, recent studies suggest these arms of the UPR are sustained in melanoma cells in response to pharmacological ER stress [15] and that this response is mediated at least in part by activation of MEK/ERK signaling [16]. MAPK signaling is particularly important for the response to ER stress, and in this context, its activation in melanoma likely maintains prosurvival UPR signaling while suppressing ER stress-induced apoptosis [17].

Other common genetic mutations in melanoma, including those that activate AKT or inhibit PTEN, may prevent excessive ER stress and thus avoid induction of proapoptotic UPR signaling by inducing a switch in metabolism from aerobic respiration to glycolysis through the activation of HKII and HIF1α [18,19]. The induction of glycolysis by cancer cells, known as the Warburg effect, fuels tumor growth in an oxygen-poor environment and has been linked to the induction of ER stress; however, through the supply of nutrients and energy, the Warburg effect likely maintains prosurvival UPR signaling and prevents the induction of apoptosis. The Warburg effect is frequently accompanied by the induction of stromal catabolism through cytokine signaling and the induction of oxidative stress in the surrounding microenvironment [20]. Cancer-induced catabolism of stromal fibroblasts, known as the reverse Warburg effect, promotes tumor growth and progression by supplying cancer cells with energy and raw materials (e.g., L-lactate, ketones, glutamine, other amino acids and fatty acids) for anabolic metabolism

via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation [21]. This may be particularly relevant for melanoma development, as patients harboring *MC1R* mutations that favor the production of pheomelanin may be susceptible to melanoma development due to the activation of stromal catabolism by pheomelanin-induced oxidative stress [22]. Furthermore, the reverse Warburg effect has been critically linked to the loss of Cav1 expression by stromal fibroblasts, which correlates with reduced survival of melanoma patients [23]. Interestingly, orthotopic melanomas in the skin of Cav1-knockout mice developed more rapidly than in wild-type mice, but the formation of lung metastases was significantly reduced, indicating Cav1 may regulate different mechanisms during melanoma tumor growth and metastatic spread [24]. In addition, ER stress within melanoma cells has been shown to suppress antitumor immunity through the paracrine-mediated inhibition of tumor-infiltrating myeloid cells, which facilitates tumor growth with fewer cytotoxic T cells [25].

Melanoma cells often have altered expression levels of ER chaperone proteins, such as Grp78/BiP and PDIs, suggesting increased basal levels of UPR signaling act to manage ER stress [26–28]. Increased Grp78/BiP expression has also been shown to correlate with melanoma progression [26], and in other cancer types has even been directly linked to increased proliferation and resistance to doxorubicin-induced apoptosis [29,30]. In addition, there are at least 21 putative PDIs in the ER, indicating that the isomerization of disulfide bonds is a specialist function. However, some PDIs appear to have extra functions, such as ERp57, which is also a chaperone-like protein that is required for the activity of the transcription factor STAT3 in melanoma [30]. Melanoma cells may become dependent upon such specialist functions, as the inhibition of ERp57 sensitizes melanoma cells to ER stress-induced apoptosis [28]. Interestingly, PDIs have also been linked to increased invasion of malignant gliomas [28], further indicating that the expression of PDIs may be linked to tumor progression.

The modulation of UPR signaling by melanoma cells may also activate a lysosomal degradation mechanism known as autophagy, which increases resistance to ER stress-induced apoptosis in response to a number of stressors, including hypoxia and vemurafenib treatment [31,32]. In this context, cytoprotective autophagy seems

to be primarily mediated via PERK signaling. Nevertheless, while the inhibition of autophagy sensitizes tumor xenografts to hypoxia and vemurafenib treatment, it remains unclear whether autophagy inhibition is a viable therapeutic strategy, as some studies have demonstrated that the induction of autophagy may actually be cytotoxic to cancer cells [33]. Furthermore, depending upon the intensity and type of stimulus, as well as microenvironmental conditions, the outcomes of autophagy activation in either normal or cancerous cells may differ.

Therefore, it remains unclear whether the UPR is beneficial or detrimental to the development of cancer, with some tumors displaying clear induction of pro-survival UPR mechanisms, while others lack UPR signaling and thus avoid the activation of ER stress-induced apoptosis.

ER stress-induced apoptosis of melanoma

It remains unclear exactly how the switch from antiapoptotic to proapoptotic UPR signaling is mediated, but in normal cells, the induction of CHOP/Gadd153 is thought to play a pivotal role. CHOP/Gadd153 reduces the transcription of the antiapoptotic protein BCL-2 while upregulating the proapoptotic BH3-only protein BIM, allowing proapoptotic BCL-2 family proteins to dominate and for apoptosis to occur [34–38]. However, in melanoma cells undergoing ER stress, PP2A is downregulated, leading to increased ERK activation, which mediates subsequent phosphorylation and proteasomal-mediated degradation of BIM [39], thereby inhibiting ER stress-induced apoptosis. Nevertheless, excessive or persistent ER stress results in the apoptosis of melanoma cells, as well as other neuroectodermal tumors, which has been critically linked to the function of ATF4, since its inhibition confers resistance to ER stress- and vemurafenib-induced apoptosis [14,40].

Calcium is a vital second messenger within the cell and a high ER/cytosol calcium gradient is critical to the induction of apoptosis. Therefore, mechanisms such as increased BCL-2 and BCL-xL expression that reduce this gradient by lowering the steady-state ER Ca^{2+} concentration reduce the potential for ER stress-induced apoptosis [41]. Melanoma cells often exploit this by increasing BCL-2 protein expression in order to favor a lower ER Ca^{2+} concentration [42], and several other types of cancer, including leukemia and breast and prostate cancer [43–45], display increased levels of the BI-1, which has

also been shown to lower steady-state ER Ca^{2+} levels and increase resistance to apoptosis [46]. As would be expected, overexpression of anti-apoptotic BCL-2 and BCL-xL or knockdown of the proapoptotic proteins BIM, PUMA and NOXA have been shown to reduce ER stress-induced cell death [35,47], including of melanoma cells [48–50]. Therefore, modulation of these pro- and anti-apoptotic proteins has also been suggested as a novel strategy for targeted melanoma therapy [36–38].

Understanding the complex molecular mechanisms that render melanoma cells resistant to ER stress-induced apoptosis will help with identifying targets that may be exploited in order to harness ER stress as a strategy to effectively treat melanoma.

Exploiting ER stress for melanoma management

Healthy cells, with the exception of major secretory cells such as B cells and gastric epithelial cells, are not normally under the influence of ER stress. This may provide a potential therapeutic window for the treatment of melanoma cells through targeted ER stress due to the lack of UPR signaling in normal cells and their high tolerance for ER stress. Strategies can be broadly divided into those that modulate UPR signaling, those that inhibit correct protein folding and those that actively induce ER stress (Table 1), all of which tip the balance of signaling in favor of apoptosis or cell cycle arrest [51]. In addition, there are also a number of therapeutic agents that induce ER stress as a side effect of other actions (e.g., the tyrosine kinase inhibitor sorafenib also induces markers of an ER stress response [52]). However, it is unclear whether the efficacy of these agents is dependent upon their ability to induce ER stress or whether melanoma cells induce a generic ER stress response as a survival mechanism.

• Inhibition & activation of UPR signaling

As PERK mediates cell cycle arrest through the inhibition of cyclin D expression and is also the major activator of CHOP/Gadd153, PERK would seem to be an ideal target for therapy. However, while experimental activation of PERK inhibits tumor growth by blocking protein translation, this also renders tumor cells more resistant to the induction of apoptosis [53]. Alternatively, the inhibition of PERK with pharmacological inhibitors such as GSK265157

significantly inhibits the growth of multiple myeloma and pancreatic cancer tumors [54] by driving proliferation while also making cancer cells more susceptible to the induction of apoptosis. Therefore, targeted inhibition of PERK may represent a potential therapeutic strategy through which to sensitize melanoma cells to ER stress-induced apoptosis.

Alternatively, targeting the IRE1 α kinase domain in order to prevent the activation of endonuclease activity and splicing of XBP1 mRNA into a prosurvival factor may also present a viable approach. However, it appears that general kinase inhibitors such as sunitinib are actually potent activators of IRE1 α endonuclease activity, as it is the conformational change due to nucleotide binding that is mimicked by the binding of the inhibitor, rather than phosphorylation itself, which is responsible for IRE1 α activation [55]. Therefore, a more promising approach may be to directly target the endonuclease activity of IRE1 α with a selective inhibitor such as STF-083010, which has already been shown to inhibit the growth of multiple myeloma xenograft tumors [56].

As for PERK and IRE1 α , the inhibition of ATF6 may also sensitize melanoma cells to ER stress. The antiretroviral drug nelfinavir, used for the treatment of HIV infection, has been shown to induce the apoptosis of liposarcoma and castration-resistant prostate cancer cells through the inhibition of S1P and S2P activity, which blocks the processing of ATF6 [57]. However, therapeutic blockade of ATF6 by 4-(2-aminoethyl) benzenesulfonyl fluoride has been shown to impair cardiac functions, leading to heart failure [58], which may severely limit the development of ATF6 inhibitors for the treatment of melanoma.

• Inhibitors of protein folding & quality control

The dependence of melanoma cells on chaperone proteins leads to ‘chaperone addiction’, whereby cells become dependent upon continual chaperone expression for survival. Therefore, inhibiting chaperone proteins is a potential strategy for the treatment of melanoma [27,59].

Inhibition of the chaperone protein Grp78/BiP by the *Escherichia coli* subtilase cytotoxin activates all arms of the UPR [60] and has also been shown to suppress prosurvival NF- κ B signaling via ATF6-dependent induction of C/EBP β and mTOR-dependent dephosphorylation of Akt [61].

Table 1. Current strategies to target endoplasmic reticulum stress for melanoma management.			
Strategy	Target	Example drug	Ref.
Inhibition of cytoprotective UPR signaling	PERK inhibition	GSK2656157	[47]
	IRE1α endonuclease inhibition	Salubrinal	[69]
		STF-083010	[49]
		MKC3946	[70]
Inhibition of protein folding and quality control	Grp78/BiP inhibition	Subtilase toxin	[27,55]
		(-)-epigallocatechingallate	[55]
		Honokiol	[55]
		Versipelostatin	[56]
	PDI inhibition	Propynoic acid carbamoyl methyl amides	[58]
		Phenyl vinyl sulfonate-containing small molecules	[59]
	HSP90 inhibition	17-AAG	[64]
		XL888	[61,62]
Induction of ER stress	Proteasome inhibition	PU-H71	[63]
		Bortezomib	[65,67]
	Modulation of ceramide metabolism	Carfilzomib	[68]
		Fenretinide	[65,71]
Combination strategies	Replication-competent oncolytic viruses	MDA7/IL-24	[75]
	PDI inhibition + ER stress inducer	–	[28,52]
	Grp78/BiP inhibition + an ER stress inducer		[55]
	Proteasome inhibition + modulation of ceramide metabolism		[65]
17-AAG: 17-Allylamino-17-demethoxygeldanamycin; ER: Endoplasmic reticulum; PDI: Protein disulfide isomerase; UPR: Unfolded protein response.			

Subtilase toxin, as well as the green tea polyphenol (-)-epigallocatechingallate and the *Magnolia grandiflora* derivative honokiol, both of which also inhibit Grp78/BiP, are potent inducers of ER stress, resulting in the apoptosis of melanoma cells [27,62]. An alternative inhibitor of Grp78/BiP is the *Streptomyces versipellis*-derived compound versipelostatin, which, rather than acting on the Grp78/BiP protein itself, blocks its expression through the activation of 4E-BP1, resulting in the downregulation of Grp78/BiP transcription [63,64].

In addition to Grp78/BiP, melanoma cells rely on PDIs in order to assist with protein folding, and as such, targeting PDIs has received much attention in recent years as a potential cancer therapy [28,59]. A class of orally bioavailable propynoic acid carbamoyl methyl amides, which inhibit PDIs, have single-agent efficacy against a panel of human ovarian cancer cell lines and may also be suitable for the treatment of melanoma [65]. However, as compounds with greater PDI specificity are created, such as the phenyl vinyl sulfonate-containing small molecules with an IC₅₀ value of around 1.7 μM [66], the role of individual PDIs will become clearer, allowing PDI inhibition to be targeted more effectively. Interestingly, PDI inhibitors have also displayed neuroprotective properties

by preventing the accumulation of mutant huntingtin and amyloid-β toxicity [67], indicating that PDI inhibitors may actually suppress the apoptosis of normal cells. However, perhaps the greatest potential of PDI inhibitors as a therapy for melanoma is to combine them with other ER stress-inducing agents [59].

The chaperone protein HSP90 is emerging as a promising target for melanoma therapy, since oncogenic BRAF^{V600E} is dependent on HSP90 for correct folding, while the folding of wild-type BRAF appears to be independent of HSP90 [68]. Therefore, while inhibiting HSP90 may be effective for a range of tumor types, the selectivity that HSP90 inhibitors appear to have for BRAF^{V600E} makes targeting HSP90 a potentially favorable therapeutic approach for the treatment of BRAF^{V600E}-mutated melanoma tumors. HSP90 inhibitors function by interfering with ATP binding and thus prevent the conformational changes that are required to correctly fold client proteins, such as BRAF^{V600E}, leading to increased ER stress and the degradation of the misfolded protein. The HSP90 inhibitor XL888 is able to resensitize vemurafenib-resistant melanoma cells by impairing the folding of those signaling proteins that are responsible for resistance, as well as by increasing BIM and decreasing Mcl1 expression [69].

Another HSP90 inhibitor, PU-H71, also results in ER stress-induced apoptosis of melanoma cells, including those that are overexpressing BCL-2 [70]. Nevertheless, as the HSP90 inhibitor 17-allylamino-17-desmethoxygeldanamycin (which is already in clinical trials) has also been shown to induce the expression of antiapoptotic HSP70 members, a strategy that combines HSP90 and HSP70 inhibitors may be more effective for the treatment of melanoma [71]. However, resistance to HSP90 inhibitors conferred by BAX deficiency may be a more difficult problem to overcome.

• Induction of ER stress

While modulating UPR signaling or interfering with protein folding may be effective against cancer cells that are already undergoing excessive ER stress, these strategies are unlikely to be effective for slow-growing, more organized tumors or tumors that undergo growth arrest. Therefore, strategies that actively induce ER stress may be a more effective therapeutic approach for some melanomas. However, the most effective approach is likely to be one that combines the inhibition of prosurvival UPR signaling with the active induction of ER stress.

Bortezomib, a US FDA-approved drug for the treatment of multiple myeloma and mantle cell lymphoma, is a cell-permeable dipeptidylboronic acid that reversibly inhibits the activity of the 26S proteasome, resulting in ER stress-induced apoptosis of a range of cancer types, including melanoma [72,73]. By blocking the function of the proteasome, bortezomib inhibits ER-associated degradation, leading to the enhanced accumulation of damaged proteins, the activation of UPR signaling and, eventually, cell cycle arrest and/or apoptosis [74]. As bortezomib is more effective against faster-proliferating cancer cells due to the more rapid accumulation of damaged proteins, it may be particularly effective for the treatment of the most aggressive melanomas. However, melanomas that are refractory to or develop resistance to bortezomib may be treated with alternative proteasome inhibitors, such as carfilzomib, which targets the 20S rather than the 26S proteasome [75]. Alternatively, the protein phosphatase inhibitor salubrinal, which prevents dephosphorylation of the PERK target eIF2 α , or the small-molecule IRE1 α endonuclease inhibitor MKC-3946, both of which have been shown to enhance the bortezomib-mediated cell death of multiple myeloma, may constitute

novel combination therapies for the treatment of melanoma [76,77].

The synthetic derivative of retinoic acid, fenretinide, also induces ER stress, resulting in the apoptosis of melanoma cells by an alternative mechanism involving the generation of reactive oxygen species and the modulation of ceramide metabolism [78,79]. Fenretinide also increases expression of the ER stress markers GRP78, PDI, calreticulin and calnexin [28]. Crucially, however, as bortezomib and fenretinide induce ER stress via different cellular mechanisms, their combined use results in the synergistic ER stress-induced apoptosis of melanoma cells [72]. Furthermore, while bortezomib and fenretinide both result in dose-dependent ER stress-induced apoptosis, their efficacy is further enhanced by the inhibition of Grp78/BiP, PDI or XIAP [27,59,80]. In addition, the efficacy of fenretinide may also be enhanced by encapsulating the drug in polymeric micelles in order to improve the delivery of the drug to the tumor [81]. Interestingly, a recent study revealed an alternative mechanism for modulating ceramide through the induction of the glycosphingolipid hydrolase GBA2, which promotes glucosylceramide degradation, resulting in the ER stress-induced apoptosis of melanoma cells [82]. Therefore, the potential may exist to develop drugs that target GBA2 in order to induce glucosylceramide breakdown as a novel way of inducing ER stress and the apoptosis of melanoma cells.

Conditionally replication-competent adenoviruses driven by the cancer-specific promoter *PEG-3* engineered to express the IL-10 cytokine family member MDA7/IL-24 eradicated primary as well as metastatic melanomas and prostate tumors in athymic mice [83] through a mechanism involving the induction of ER stress, indicating that this strategy may be a potential therapeutic option for melanoma. MDA7/IL-24 has recently been associated with pronounced ER stress-induced apoptosis of acute myeloid leukemia cells, breast cancer-initiating cells and neoplastic epithelial cells, while exerting minimal toxicity towards the normal counterparts [84,85]. The efficacy of MDA7/IL-24 requires the downregulation of antiapoptotic MCL-1 and is therefore markedly enhanced by its combination with the MCL-1-targeting inhibitor BI-97C1 (sabutoclax, which also has high affinity for BCL-2, BCL-XL and BFL-1) [86]. Furthermore, MDA7/IL-24 efficacy is also enhanced by cotreatment with a secretable form of the ER-resident chaperone

Grp70, which stimulates a systemic antibody-mediated immune response [87]. Interestingly, however, lethality in response to MDA7/IL-24 was abrogated in PERK^{-/-} cells [88], indicating that the efficacy of MDA7/IL-24 requires PERK-mediated signaling, probably via ATF4, in order to induce apoptosis.

Conclusion & future perspective

Melanoma evolves with a repertoire of mutations that makes them particularly resistant to the induction of apoptosis. Many studies have shown that activated UPR signaling, rather than impaired UPR signaling, correlates with tumor progression. However, it is unclear whether this is a consequence of elevated ER stress due to mutational and environmental pressure or whether prosurvival signaling is actively elevated as a consequence of mutations that commonly arise in melanoma.

Therapeutic strategies that rely on the induction of ER stress in order to tip the balance of UPR signaling in favor of apoptosis are promising. However, as the homeostatic response to ER stress activates prosurvival signaling, this strategy relies on the sufficient induction of ER stress in order to induce tumor cell death while avoiding the induction of pseudosenescence and its associated side effects. The most effective strategy for exploiting ER stress for melanoma therapy will probably involve the combination of agents that are able to inhibit cytoprotective UPR signaling with those that actively induce ER stress. A further novel therapeutic approach may be to metabolically uncouple anabolic cancer cells from their glycolytic stroma using inhibitors of glycolysis, such as the synthetic glucose analog 2-deoxy-D-glucose, in combination with an ER

stress-inducing agent in order to drive proapoptotic signaling. Furthermore, the development of the HSP90 inhibitor 17-allylamino-17-desmethoxygeldanamycin demonstrates the feasibility of targeting the intrinsic ATPase activity of molecular chaperones, and this strategy may be exploited for the inhibition of other chaperones, including Grp78/BiP and Grp94, which also rely on the hydrolysis of ATP. Finally, the activation of the immune system is likely to be required for the complete clearance of tumors, and as such, the combination of ER stress-inducing agents with activators of the antitumor immune response, such as CTLA-4- or PD-1-blocking antibodies, may be of therapeutic benefit. Ongoing trials will thus pave the way for the development of more effective therapeutic strategies that harness ER stress for the treatment of melanoma.

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