PERSPECTIVES

Treatment combinations targeting apoptosis to improve immunotherapy of melanoma

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Abstract Immunotherapy based on T cell responses to the tumor is believed to involve killing of cancer cells by induction of apoptosis. The predominant mechanisms are death ligand-induced signaling mainly by TNF-related apoptosis-inducing ligand (TRAIL) mediated by CD4 T cells, monocytes and dendritic cells, and perforin/granzyme mediated apoptosis mediated by CD8 T cells and NK cells. Resistance against TRAIL involves loss of TRAIL death receptors and/or activation of the MEK and/or Akt signal pathways. Resistance to CD8 CTL responses also involves activation of the MEK and/or Akt pathways. Apoptosis induced by immune responses is regulated by the Bcl-2 family of proteins. Many reagents have been developed against the Bcl-2 antiapoptotic proteins and clinical trials combining them with immunotherapy are awaited. The second group of agents that regulate the Bcl-2 family of proteins are the signal pathway inhibitors. Clinical trials with inhibitors of RAS, RAF or MEK are in progress and would appear an exciting combination with immunotherapy. One of the main drivers of resistance to apoptosis are adaptive mechanisms that allow cancer cells to overcome endoplasmic reticulum (ER) stress. These adaptive mechanisms inhibit practically all known apoptotic pathways and create an acidic environment that may reduce infiltration of lymphocytes against the tumor. The signal pathway inhibitors may be effective against these adaptive processes but additional agents that target ER stress pathways are in development. In conclusion, combination of immunotherapy with agents that target antiapoptotic mechanisms in cancer cells offers a new approach that requires evaluation in clinical trials.

Keywords Apoptosis · Bcl-2 proteins · Signal pathway inhibitors · Endoplasmic reticulum stress

Introduction

Immunological responses are believed to play a role in the natural history of cancers such as melanoma and can be demonstrated in immunohistological studies and by a variety of assays carried out ex vivo on lymphocytes from patients. Evidence from these sources has prompted clinical trials with vaccines using whole cells or cell lysates or more purified antigens known to be recognized by the immune system. The results of these studies have generally been disappointing, as reviewed elsewhere [47, 103, 125].

These results have posed somewhat a dilemma as in many of the studies ex vivo assays have reported the induction of lymphocyte responses against the tumor but with little or no clinical benefits. Various explanations have been proposed to account for this lack of correlation, such as inhibition of antigen presentation, inhibition of cytokine production from T cells, anergy of T cells due to lack of costimulatory CD80, CD86 ligands, or interaction with PD1 ligands, induction of T regulatory cells, shift of T cell populations to TH2 or TH17 helper cells due to cytokines from tumor cells such as IL-6, TNF-α, IL-23, inhibition of leukocyte migration due to release of VEGF or PGE2 from tumor cells, selection of MHC and antigen loss variants [49]. Good studies and models supporting these explanations are numerous. To a large extent, however, they are

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focused on defects in the immune system and may be overlooking more fundamental properties of tumor cells that limit the effectiveness of immunotherapy even in the face of otherwise strong immune responses.

One of these properties is resistance to apoptosis, which is regarded as important as dysregulated cell division in development of tumors [41]. Given that the immune system kills tumor cells by induction of apoptosis, combining immunotherapy with agents that overcome apoptosis resistance pathways would appear a logical step in treatment. In the sections below, we indicate the principal mechanisms involved in resistance of cancer cells to apoptosis and new agents that may be effective in overcoming the resistance pathways.

How lymphocytes kill tumor cells

In general, there are two main killing pathways. The death ligand route utilized by CD4 T cells, monocytes and dendritic cells (DCs) involves interaction of members of the TNF family with their receptors in cell membranes of the cancer cells, which results in aggregation of the receptors, binding of adapter proteins such as Fas associated death domains (FADD) and activation of apical caspases such as caspase 8 through death effector domains (DED) [8, 9]. In some sensitive lymphoma cells the caspase cascade activated by caspase 8 can lead to direct activation of the effector caspases such as caspase 3 but in most solid cancers the pathway involves cleavage of the proapoptotic BH3 (Bcl-2 homology domain 3) protein Bid, which leads to activation of Bax/Bak proteins and mitochondrial outer membrane permeabilization (MOMP). This leads to release of aptogenic proteins from mitochondria and activation of the mitochondrial apoptosis pathway involving cytochrome c, APAF-1 and caspase 9, leading to formation of the apoptosome and activation of the effector caspases 3 and 7 [48]. This process is also facilitated by release of proteins such as Smac/DIABLO, which inhibit "inhibitor of apoptosis proteins" (IAPs), particularly XIAP, which inhibits events downstream of mitochondria [140].

The other main route utilized by CD8, CTL and NK cells depends on the transfer of cytotoxic granules into the immunological synapse formed between the effector and tumor cell. Proteases called granzymes are released from the granules and delivered to the tumor cell by perforin, which is a Ca^{2+}-depending pore forming protein [14, 123], which multimerizes in the cell membrane [96]. This results in cleavage of substrates such as Bid by Granzyme B, resulting in MOMP and the events described above downstream from mitochondria that lead to apoptosis. This is an oversimplified view of killing by CD8 CTL in that granzyme B may also trigger other pathways to apoptosis such as cleavage of caspase 3 and 7 [131]. Additional granzymes

such as granzyme A may contribute to cell death either by generation of reactive oxygen species (ROS) or by cleavage of proteins in the SET complex that leads to single strand breaks in DNA[123], leading to activation of p53. Killing by CTL appears to involve p53, which would be consistent with the above [87]. Granzymes H and K are called orphan enzymes as their substrates are unknown. They and granzyme M cause apoptosis by caspase-independent pathways [135].

Inhibitors of the cell death pathways

Mitochondrial dependent apoptotic pathways are regulated mainly by the Bcl-2 family of proteins, which, as reviewed elsewhere [21, 39, 51, 132] consist of a family of BH3 only proapoptotic proteins, two multidomain proapoptotic proteins (Bax and Bak) and several multidomain antiapoptotic proteins (Bcl-2, Bcl-XL, Bcl-w, Mcl-1 and A1) (see Fig. 1). In one model the antiapoptotic proteins bind the BH3 proteins and this displaces Bax or Bak from the antiapoptotic proteins, allowing them to bind to mitochondria and induce MOMP [18, 132]. Certain of the BH3 proteins have selectivity for different antiapoptotic proteins. In particular, Noxa binds selectively to Mcl-1. The latter also binds Bak and hence Noxa may displace Bak from Mcl-1, allowing it to bind to mitochondria [19, 133].

These findings are of particular interest in that immuno-histological studies on tissue sections from melanoma have shown that Mcl-1 and Bcl-XL increase in expression with progression of the disease whereas Bcl-2 decreased during progression of the disease [142]. The factors determining the changes in the antiapoptotic proteins are not entirely clear but Mcl-1 and Bcl-XL appeared inversely correlated with expression of the transcription factor AP-2 and was
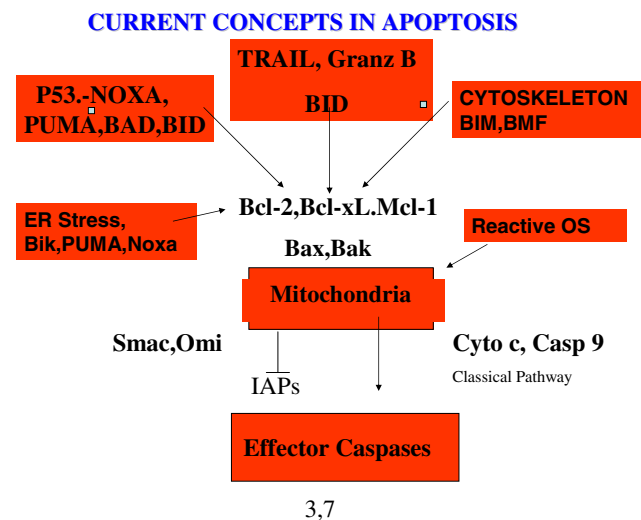

Fig. 1 Current views of how the mitochondrial pathway to apoptosis is regulated

weakly associated with Stat 3 expression. Further studies are needed to more closely define the regulators of these proteins, particularly Mcl-1 as current studies suggested it is upregulated as part of the unfolded protein response (UPR) to endoplasmic reticulum (ER) stress and is a major adaptive mechanism that prevents ER stress-induced apoptosis [50]. We [137] and others [70, 128] have also shown Mcl-1 is a critical factor in resistance to TNF-related apoptosis-inducing ligand (TRAIL).

These findings with respect to Mcl-1 in melanoma are important in design of treatment strategies in melanoma. As shown in Table 1, there are now a number of new agents that can be used clinically to target the antiapoptotic proteins. One of these is the Abbott ABT 737 agent, which has high affinity for Bcl-2 and Bcl-XL and Bcl-W. Preclinical studies have shown that many tumors were resistant to this agent due to Mcl-1 proteins in the tumor. Downregulation of Mcl-1 resulted in sensitivity to ABT 737 [20, 118]. These results are relevant to immunotherapy, particularly as ABT 737 was shown to sensitize tumor cells to killing by CTL [74]. ABT 263 is an orally active form of ABT 737 [116]. As shown in Table 1, however, there are a number of small mol. wt. inhibitors of the antiapoptotic proteins that have selectivity for all the antiapoptotic proteins, e.g. Obatoclax is now in preliminary trials in patients with hematological malignancies [105, 114]. At this stage, we would expect that these broad spectrum inhibitors would potentiate the effects of various forms of immunotherapy.

Death ligand mediated killing: the Yin Yang of TRAIL death receptor signals

In the special case of TRAIL, the level of expression of death receptors, TRAIL-R1 and R2 were found to be major determinants of their susceptibility to killing by TRAIL [138]. Furthermore, progression of melanoma was associated with downregulation of receptors [143]. The cause of receptor downregulation is unknown but it is clear that immunotherapy that depends on administration of TRAIL or TRAIL mediated killing by CD4 T cells, DCs and monocytes, would be ineffective against TRAIL-R negative cancers. Upregulation of death receptors in some cancers can be achieved by chemotherapy that targets DNA and is p53 dependent [26]. However, in melanoma a wide range of chemotherapy agents and signal pathway inhibitors did not upregulate death receptors. As reported in studies on colon carcinoma cells [65] and prostate carcinoma [108], the ER stress inducer Tunicamycin was able to do so [63]. This was related to induction of the UPR response to stress. The transcription factor CCAAT/enhancer-binding protein homologous protein (CHOP) (Gadd153) was involved at late stages of receptor upregulation (36 h) but other factor(s) were involved at earlier periods [63]. Tunicamycin is considered too toxic for clinical use but several other agents that are in clinical use, such as Cox 2 inhibitors [68] or Dipyridamole, may be useful for this purpose [38]. They and curcumin from curry powder appear to act on CHOP to upregulate receptors [66].

One of the peculiarities of the TRAIL system is the concurrent delivery of opposing death and survival signals from its receptors [Yin (negative) and Yang (Positive)]. It has been known for some time that TRAIL receptors may associate with other adaptor proteins rather than or in addition to FADD and result in different outcomes rather than cell death. Principal among these is activation of NF-κB and JNK most likely through the receptor interacting protein (RIP) and TNF receptor associating factor 2 (TRAF2) [25]. We and others have shown that activation of NF-κB in melanoma by TRAIL is strongly anti-apoptotic [33]. One of the consequences of activation of NF-κB and of Akt [93] is upregulation of Flice inhibitory protein (cFLIP), which can bind to DED of FADD and caspase 8 and inhibit

Table 1 Targeting anti-apoptotic proteins

Agent	Target protein(s)	Trial stage	References
Oblimersen (G3139)	Bcl-2 (specific)	Stage III	Bedikian et al. [13]
YM155	Survivin	Phase II	Iwasa et al. [62]
LY2181308 (antisense)	Survivin	Phase II	Hunter et al. [58]
ABT-737 (ABT-263)	BH3-mimetic (inhibits Bcl-2 group: Bcl-2, Bcl-XL, Bcl-W, not Mcl-1)	Phase I	Chen et al. [20], Tse et al. [116], van Delft et al. [118]
Gossypol (AT-101)	BH3-mimetic (inhibits Bcl-2 group)	Phase I	MacVicar et al. [82]
Obatoclax (GX015-070)	BH3-mimetic (inhibits Bcl-2 group)	Phase I/II	Schimmer et al. [105]
SAHB	BH3 mimetic	Preclinical	Pitter et al. [97]
TW37	Bim-mimetic (inhibits Bcl-2 group)	Preclinical	Wang et al. [130]
Smac mimetics	Inhibitor of IAP$_{1,2}$, XIAP	Preclinical	Zobel et al. [144]
Smac mimetic SM-164	Inhibitor of IAP$_{1,2}$, XIAP	Preclinical	Lu et al. [78]
Smac mimetic Smac037	Inhibitor of IAP$_{1,2}$, XIAP	Preclinical	Cossu et al. [22]

apoptosis. cFLIP may also bind to TRAF1, 2 and RIP, resulting in activation of NF-κB and ERK1/2 [122, 134]. Inhibitors of apoptosis proteins 1 and 2 (IAP1, 2) were also shown to be important in the activation of NF-κB (via ubiquitin domains) [43] by TNF-α and when IAPs were inhibited, activation of NF-κB did not occur [120]. Smac mimetics were shown to result in TNF-induced apoptosis by activation of caspase 8 either by inhibition of c-FLIP production or formation of a RIP/FADD/caspase 8 complex [127]. These observations have led to the development of a number of new agents, which target IAP1, 2 and XIAP, as shown in Table 1 [22, 78, 127, 144].

In addition, the MEK pathway may be strongly activated by TRAIL. This is rapid but relatively transient, peaking at 1 hour after exposure to TRAIL [137]. Activation of MEK is dependent on activation of protein kinase C (PKC), particularly PKC epsilon (ε), and the sensitivity of melanoma cells to TRAIL is inversely related to the activation of PKC-ε [37]. Activation of PKC is upstream of RAF but whether it is activated directly by TRAIL or by phospholipase C, as described for TNF-α [12], is not clear. The activation of MEK by TRAIL occurs irrespective of whether BRAF is mutated or not [137].

These results therefore imply that within a polyclonal population of melanoma cells there is a range of activation signals in response to TRAIL. Sensitive cells have predominant activation of the FADD/caspase 8 pathway whereas resistant cells have dominant activation of NF-κB and MEK pathways. These results clearly have implications in selecting treatment combinations.

Table 2 summarizes some of the experimental studies on combinations with TRAIL that may increase apoptosis. In general, they can be viewed as agents which upregulate TRAIL-R1–R2 receptors or which down-regulate anti-apoptotic proteins. Bortezomib appears to mediate its effect by down-regulation (directly or indirectly) of anti-apoptotic proteins such as cFLIP, Mcl-1 and NF-κB and upregulation of Noxa [90]. Clinical experience with the drug is mainly

limited to hematologic malignancies such as multiple myeloma and mantle cell lymphoma, for which it has received FDA approval. Histone Deacetylase inhibitors have had relatively little effects when used as single agents but may be most effective when used as sensitizing agents to induce apoptosis [81, 139].

Signal pathway inhibitors as partners for immunotherapy

Two survival pathways: the MEK/ERK and PI3-K Akt pathways are frequently upregulated in cancer cells [83, 107], as reported in melanoma [137, 141] and are potent inhibitors of apoptosis. We and others have shown that inhibition of the MEK/ERK pathway sensitizes cancer cells to TRAIL-induced apoptosis [137]. Similar results were obtained with inhibitors of Akt and when both inhibitors were used the results were additive [110]. In short-term assays inhibitors of the MEK pathway did not induce apoptosis when used alone but studies over longer periods resulted in killing of approximately 50% of melanoma lines due to upregulation of PUMA and Bim, and downregulation of Mcl-1 [129]. The multiple sites of action of the MEK pathway that inhibit apoptosis are shown in Table 3 and reviewed elsewhere [10]. The Akt pathway also has multiple sites of action that inhibit apoptosis, as shown in Table 4 from the review by Manning and Cantley [83].

Table 3 The ERK1/2 pathway blocks apoptosis at multiple sites

Inhibits Bim EL by phosphorylation Ser 69 and other sites [10, 17]

Phosphorylates Bad indirectly via RSK [10]

Repression of Bmf translocation [119]

Induces Mcl-1 [10, 129]

Induces GRP78-(GRP78 binds Bik, caspase 4) [63, 64]

Induces IL-8 and upregulation of ICAM [73]

Increases HIF-1A expression [75]

Table 2 Treatment combinations with TRAIL or agonistic antibodies to TRAIL

Agent	TRAIL/A.MAbs	Cancer	Mechanisms of action	References
Cox-2 inhibitors	TRAIL	Hepatocellular Ca	↑TRAIL R1, R2 ↓Mcl-1	Kern et al. [68]
Dipyridamole	TRAIL	Colon and prostate Ca	CHOP mediated ↑TRAIL R1, R2	Goda et al. [38]
Curcumin	TRAIL	Renal Ca	↑DR5	Jung et al. [66]
Bortezomib	A.Mabs	NSCLC	↓Mcl-1, ↓FLIP	Luster et al. [80]
Quercetin	TRAIL	Colon Ca	TRAIL R1, R2 in lipid rafts	Psahoulia et al. [98]
Sodium arsenite	TRAIL	Melanoma	TRAIL R1, R2↑, cFLIP↓	Ivanov et al. [61]
Resveratol	TRAIL	Melanoma	NF-κB↓, Stat3↓, cFLIP, Bcl-XL	Ivanov et al. [60]
Vorinostat (HDACi)	MAb MD5 (murine)	Mouse breast Ca	cFLIP↓	Frew et al. [34]
Quercetin	TRAIL	Human hepatoma	DR5↑, cFLIP↓	Kim et al. [70]
Triterpenoid CDOO-Me	TRAIL	Human lung cancer	cFLIP↓ degradation	Zou et al. [145]

Table 4 Inhibition of apoptosis by activated Akt [83]

Phosphorylates FOXO transcription factors resulting in decreased Bim
Phosphorylates and inhibits BAD
Phosphorylates and inhibits GSK3b
Activates NF-kB
Activates HDM2 and thereby decreases p53

MEK inhibitors or inhibitors of Ras and RAF upstream of MEK may therefore help in sensitizing melanoma cells to immunotherapy [126]. They may have other unexpected benefits in that MEK inhibitors were shown to upregulate melanoma differentiation antigens [71] and inhibit release of immunosuppressive factors IL-10, VEGF and IL-6 from melanoma [113]. RAF knockdown was shown to reduce ICAM-1 expression and IL-8 production from melanoma and to inhibit extravasation through blood vessels [73].

Table 5 shows the many RAS/BRAF/MEK signal pathway inhibitors are now available. They are reviewed in detail elsewhere [126] and as combinations with other therapies [112]. It is unknown whether they may have deleterious effects on immune function due to direct effects on lymphocytes. Relatively few studies have been conducted on their effects on the immune system but in this regard the RAF inhibitor PLX4032 (that is specific for mutated BRAF) should not have targets in lymphocytes. Holmstrom et al. [55] speculated the MEK pathway may prevent T cells from undergoing activation-induced death. Consistent with this, NRAS activating mutations were reported to be associated with autoimmune disease [91]. Sorafenib was found to inhibit the generation of vaccine specific CD8 T cells in mice. Sunitinib did not have these suppressive effects [52].

Development of inhibitors of Akt signaling is at an early stage (Table 6) and is discussed elsewhere [29, 59, 81]. The PI3K pathway was identified as critical for induction of a proliferative response to IL-2 in lymphocytes [16]. Treatment with an mTOR inhibitor, Rapamycin, was reported to sensitize cancer to adoptive immunotherapy in vivo [44], which is somewhat surprising given the role of Rapamycin as an immunosuppressive agent. It is highly likely that the effects of these drugs on immune responses will need to be evaluated in each system.

Adaptation to ER stress as the driver of resistance to immunotherapy

Under stress conditions such as hypoglycemia or hypoxia, proteins in the ER may not undergo folding or glycosylation and accumulate as unfolded proteins. These bind to a chaperone protein called glucose-regulated protein 78 (GRP78), which is normally bound to the intraluminal domains of three sensor proteins called inositol-requiring transmembrane kinase and endonuclease 1α (IRE1α), activation of transcription factor 6 (ATF6), and protein kinase-like ER kinase (PERK). Release of these proteins results in their activation and initiation of three signal pathways called the UPR. As an adaptive response, the UPR is activated to alleviate the stress condition imposed on the ER and is orchestrated by transcriptional activation of multiple genes mediated by IRE1α and ATF6, a general decrease in initiation of translation and selective translation of specific mRNAs mediated by PERK. However, if the stress on ER remains unresolved, prolonged activation of the UPR can lead to apoptosis [69].

This is the outcome in normal cells but even under extreme ER stress cancer cells survive due to adaptive processes that are as yet not fully understood. Activation of the MEK/ERK and Akt pathways are involved [57, 63]. Induction of the chaperone protein GRP78 may sequester one of the BH3 proteins in the ER called Bik [35]. GRP78 also sequesters caspase 4 [63]. HDM2 becomes activated during ER stress either via Akt activation or direct phosphorylation

Table 5 RAS/RAF/MEK signal pathway inhibitors

Agent	Class of inhibitor	Target protein(s)	References
Sorafenib	Multikinase inhibitor	C-Raf; B-Raf; VEGF-2, -3; PDGF; Flt-3; c-Kit	Amaravadi et al. [2], Flaherty [31], Hauschild et al. [46], McDermott et al. [86]
Tanespimycin (KOS-953, 17-AGG)	Hsp90 inhibitor	Hsp90 (client proteins B-Raf, Akt, others)	Kefford et al. [67]
RAF265	Multikinase inhibitor	Mutant B-Raf, VEGFR-2	Fecher et al. [29]
PLX4032	Selective B-Raf kinase inhibitor	Mutant B-Raf	Tsai et al. [115]
PD0325901	Non-ATP-competitive specific MEK inhibitor	MEK1, 2	Fecher et al. [29]
AZD6244	Non-ATP-competitive specific MEK inhibitor	MEK1, 2	Dummer et al. [24]
Tipifarnib (R115777)	Farnesyl transferase inhibitor	Prenylated proteins	Fouladi et al. [32], Haluska et al. [45]

Table 6 Akt, receptor tyrosine kinase (RTK), and Stat signal pathway inhibitors

Agent(s)	Target protein	References
SF1126 (LY294002-prodrug)	PI3K	Garlich et al. [36]
Perifosine, PX-866	Akt	Lopiccolo et al. [77]
CMEP	Akt	Zhang et al. [136]
GSK 690693	Akt	
Temsirolimus (CCI-779)	mTOR	Abraham and Eng [1]
Everolimus (RAD001)	mTOR	Abraham and Eng [1]
Deforolimus (AP23573)	mTOR	Mita et al. [88]
XL765	P13K/mTOR	Papadopoulos et al. [94]
PI 103	PI3K/mTOR	Raynaud et al. [102]
SB216763, DW1/2	GSK3β	Smalley et al. [111]
Imatinib, dasatinib, sunitinib, erlotinib	RTKs	Hodi et al. [53]
Dasatinib	Src	Guilhot et al. [42]
S31-M2001	Stat3	Siddiquee et al. [109]
SUI1274	c-Met/HGF	Natali et al. [89], Puri et al. [99]

by PERK [11]. It is possible but not yet proven that this may account for low p53 levels in some cancers such as melanoma. In addition to these direct effects on apoptosis, ER is also associated with metabolic changes resulting in glycolysis and lactic acid production [40, 92]. This results in an acidic environment that may inhibit the function or entry of immune cells into the tumor micro-environment. Table 7 summarizes some of the known antiapoptotic effects of ER stress.

Agents that may reduce the effects of ER stress on resistance to apoptosis

Given the importance of ER stress in resistance to immunotherapy, agents that overcome the adaptive pathways may be important to combine with immunotherapy. We know from studies in vitro that MEK inhibitors may overcome resistance to ER stress-induced apoptosis most probably by downregulation of GRP78, which is a target for this pathway. One of the consequences of GRP78 downregulation is release of caspase 4 (that is normally bound by GRP78) so resulting in apoptosis of some melanoma [63]. Another

Table 7 ER stress induces antiapoptotic effects [50]

Upregulation of Bcl-XL, Mcl-1. Downregulation of Bcl-2

Activation of Akt, MEK/ERK

Upregulation of GRP78

Downregulation of p53 (via ?HDM2)

Glycolysis and acidification of the microenvironment

approach is development of drugs that inhibit the activity of IRE1α so interfering with some of the downstream effects of the UPR. One such drug is referred to as Irestatin 9389 [30] (Table 8).

Novel agents that target GRP78, such as epigallocatechin, are reviewed elsewhere [72, 100]. Other approaches involve targeting more downstream effects of the UPR such as upregulation of p53 by agents which interfere with binding of p53 with HDM2, such as Nutlin 3a [121]. Inhibitors of GSK3β, such as the organometallic protein kinase inhibitor DW1/2, were also shown to upregulate p53 perhaps due to effects on HDM2 [111] or by inhibiting the phosphorylation of p53 by GSK3β [101]. GSK3β inhibitors may, however, increase the stability of Mcl-1 as the latter was shown to be a target for phosphorylation by GSK3β in IL-3 dependent cells [85]. GSK3β inhibitors were also reported to increase TRAIL death receptor expression by activation of c-MYC [104].

Drugs that might exploit the metabolic consequences of ER stress such as excess lactate production may have a role in combination with immunotherapy. These include proton pump inhibitors such as Omeprazole, which target H(+)-ATPases [23] and monocarboxylate transporters (MCT) 1–4 [15, 84], as well as Na$^+$/H$^+$ exchangers. MCT transporters such as MCT-1 appeared more important in regulation of pH in melanoma cell lines [124] than Na$^+$/H$^+$ exchange pumps. MCT isoforms may, however, have opposing roles as studies on human cervical carcinoma suggested that MCT-4 transported lactate out of cells whereas MCT-1 transported lactate into cells where it was a source of energy under aerobic conditions [106]. Omeprazole was shown to increase the sensitivity of xenografts to cisplatin and this combination is the subject of ongoing clinical studies in melanoma.

Induction of ER stress is also believed to result in upregulation of heme oxygenase-1 (HO-1) due to induction of ROS [76] and has a cytoprotective effect. There is limited information about its role in protection of melanoma against these products of ER stress but inhibitors of HO-1 such as zinc protoporphyrin and polymeric derivatives [27] may have a role in increasing apoptosis to ER stress-inducing agents [28].

Table 8 Additional agents to target ER stress-induced resistance to apoptosis [50]

Agents that target HDM2 and increase p53, e.g. Nutlin 3a

Inhibitors of GSK3β that target S 315,376 on p53, DW 1/2

Inhibitors of GRP78, IRE1α (epigallocatechin, Irestatin)

VEGF-R2 inhibitors (AZD 2171), proton pump inhibitors (Omeprazole)

Monocarboxylate transporter (MCT) inhibitors

Zinc protoporphyrin inhibitors of HO-1

Targeting anti-apoptotic proteins for destruction by the immune system

Much interest also exists in decreasing the resistance of tumor (melanoma) cells to apoptosis by inducing T cell responses against the proteins concerned. This subject is well reviewed elsewhere [4]. In particular, several members of the IAP family were shown to be targets of the immune system. One of these, survivin, was referred to as a universal tumor antigen [7] and both MHC class I [54] and class II [95] epitopes identified. Phase I studies against survivin have been conducted or are in progress in patients with bladder cancer and melanoma [3, 56] and breast cancer [117]. T cell responses against ML-IAP (Livin) were also reported to be common in melanoma patients. Spontaneous CTL responses have also been identified against Mcl-1 [6] and Bcl-XL [5], indicating that these two proteins may also be effective targets for immunotherapy. It is also possible that immunotherapy against Mcl-1 may be complementary to treatment with ABT-737, which is limited in effectiveness due to lack of specificity against Mcl-1 (see above).

Conclusion

Increased knowledge concerning cell death pathways used by lymphocytes to kill cancer cells and the availability of many new agents that can inhibit resistance pathways against apoptosis have set the scene for new approaches to immunotherapy based on combinations with the new agents. This includes immunotherapy, not only with vaccines but logically also with adoptive immunotherapy and CTLA4 antibodies. Several agents appear particularly promising, e.g. agents which directly target the anti-apoptotic proteins in melanoma would appear to provide much scope for sensitization of melanoma to immunotherapy but it is also possible that inhibitors of signal pathways, which regulate expression of these proteins may be equally effective. Similarly, agents such as Bortezomib and Histone deacetylase inhibitors may find their true role as sensitizers of melanoma to immunotherapy. Evaluation of these drugs in patients undergoing immunotherapy with vaccines, cytokines, adoptive immunotherapy or CTLA4 antibodies in well-planned clinical trials is now needed.

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