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Chemosensitization of Prostate Cancer by Modulating Bcl-2 Family Proteins

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Abstract

A major challenge in oncology is the development of chemoresistance. This often occurs as cancer progresses and malignant cells acquire mechanisms to resist insults that would normally induce apoptosis. The onset of androgen independence in advanced prostate cancer is a prime example of this phenomenon. Overexpression of the pro-survival/anti-apoptotic proteins Bcl-2, Bcl-xL, and Mcl-1 are hallmarks of this transition. Here we outline the evolution of therapeutics designed to either limit the source or disrupt the interactions of these pro-survival proteins. By either lessening the stoichiometric abundance of Bcl-2/xL/Mcl-1 in reference to their pro-apoptotic foils or freeing these pro-apoptotic proteins from their grip, these treatments aim to sensitize cells to chemotherapy by priming cells for death. DNA anti-sense and RNA interference have been effectively employed to decrease Bcl-2 family mRNA and protein levels in cell culture models of advanced prostate cancer. However, clinical studies are lagging due to *in vivo* delivery challenges. The burgeoning field of nanoparticle delivery holds great promise in helping to overcome the challenge of administering highly labile nucleic acid based therapeutics. On another front, small molecule inhibitors that block the hetero-dimerization of pro-survival with pro-apoptotic proteins have significant clinical advantages and have advanced farther in clinical trials with promising early results. Most recently, a peptide has been discovered that can convert Bcl-2 from a pro-survival to a pro-apoptotic protein. The future may lie in targeting multiple steps of the apoptotic pathway, including Bcl-2/xL/Mcl-1, to debilitate the survival capacity of cancer cells and make chemotherapy induced death their only option.

Keywords

Chemoresistance; prostate cancer; Bcl-2; apoptosis; BH3 mimetics; Mcl-1

INTRODUCTION

Failure to respond to chemotherapy, or chemoresistance, represents a critical problem in the treatment of prostate cancer. Chemosensitizing cancer cells by incorporating a new component into current cancer chemotherapy would have immense clinical relevance and invaluable benefit to prostate cancer patients. One major mode by which cancer cells evade chemotherapy is by an acquired ability to suppress apoptosis. The Bcl-2 family members are important regulators of apoptosis (programmed cell death) and are implicated in prostate cancer chemoresistance. In this review, we discuss the roles of Bcl-2 family proteins and

apoptosis in prostate cancer chemoresistance, focusing on the anti-apoptotic Bcl-2 domain proteins as therapeutic targets plus innovations in developing novel and more effective molecular therapies for human prostate cancer.

CURRENT THERAPEUTIC STRATEGIES ARE NOT VERY EFFECTIVE FOR THE TREATMENT OF ADVANCED, ANDROGEN-INDEPENDENT PROSTATE CANCER

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related death in North American men. Androgen deprivation therapy (ADT) is the cornerstone treatment for men with *de novo* or recurrent metastatic prostate cancer [1]. Unfortunately, androgen deprivation therapy is primarily palliative, with nearly all men progressing to an androgen-independent (AI) state [1]. Current therapeutic strategies are not very effective for treatment of advanced, androgen-independent prostate cancer. Despite several hundred clinical studies of both experimental and approved chemotherapeutic agents, chemotherapy has limited anti-tumor activity, with an objective response rate of less than 50% and no demonstrated survival benefit [2]. Thus, androgen-independent disease is the main obstacle to improving the survival and quality of life in patients with advanced prostate cancer. Considerable effort has been focused toward developing novel therapeutic strategies for treatment of advanced prostate cancer by specifically targeting the fundamental molecular basis of progression to androgen-independence and resistance of androgen-independent disease to chemotherapy.

ACQUIRED RESISTANCE TO APOPTOSIS IS A MAJOR OBSTACLE IN CANCER THERAPY

Apoptosis or programmed cell death is a mode of cell death and is important for normal development, host defense and suppression of oncogenesis [3,4]. Apoptosis not only plays an important role in tissue sculpting during development, but is also the primary defense against cells that may pose a threat to the well-being of the whole organism [5]. Faulty regulation of apoptosis has been implicated in cancer, degenerative conditions and vascular diseases [6,7]. Normal tissue is maintained by a fine balance between cell proliferation and apoptosis, and defects in apoptosis play an important role in carcinogenesis and tumor progression [7,8].

Most anticancer therapies work by inducing apoptosis in cancer cells. The aggressive cancer-cell phenotype is the result of a variety of genetic and epigenetic alterations leading to dysregulation of intracellular signaling pathways, including cell-death signaling [9]. Lack of appropriate apoptosis due to defects in the normal apoptosis machinery plays a crucial role in resistance to a wide variety of current anticancer drugs [4,8]. For example, primary or acquired resistance of hormone-refractory prostate cancer to current treatment protocols has been associated with apoptosis-resistance of cancer cells and is linked to therapeutic failure [7,10,11]. Current and future efforts toward designing new modalities to improve survival and quality of life for cancer patients must include strategies that specifically target cancer-cell resistance to apoptosis [7,8,10].

THE BCL-2 FAMILY CONTROLS CELL DEATH VIA INTERACTIONS BETWEEN BCL-2 HOMOLOGY DOMAINS

Bcl-2 is the founding member of a family of proteins associated with cell death signaling and was first isolated as the product of an oncogene [12,13]. This family of proteins now includes both anti-apoptotic molecules such as Bcl-2, Bcl-xL, and Mcl-1, and pro-apoptotic

molecules such as Bax, Bak, Bim, Bid and Bad [14]. These proteins mainly regulate apoptosis at the mitochondrial outer membrane and control the initiation of MOMP (mitochondrial outer membrane permeabilization) [12,15,16]. A detailed description of the binding interactions and requirements has been reviewed extensively elsewhere [17-19]. However, a cursory overview of several of the particulars require mention here. Briefly, Bcl-2 family proteins are so named due to the appearance of up to four regions of sequence homology dubbed Bcl-2 homology (BH1-4) domains. Structural studies of the pro-survival proteins (Bcl-2, Bcl-xL, Bcl-w, and Mcl-1) reveal that three of these domains (BH1-3) arrange to form a hydrophobic binding pocket where BH3 domains of either BH3 only proteins (Bim, Bad, Puma, Noxa, etc.) or the canonical pro-apoptotic proteins Bax/Bak can bind. This binding groove has been the focus of innumerable efforts to develop BH3 mimetics (discussed in detail below) that could inhibit the interaction of pro-survival proteins with their cognate pro-apoptotic partner thus inducing apoptosis.

Understanding the altered stoichiometry of Bcl-2 family members in malignant cells has emerged as one of the most important considerations in designing effective treatment. Moreover, many chemotherapeutic regimens further alter pro-survival/pro-apoptotic protein ratios by inducing expression of pro-apoptotic proteins, but in resistant cells, compensatory mechanisms negate this effect. For example, a transforming mutation in Ras can thwart the Bim accumulation stimulated by paclitaxel because Bim is phosphorylated and targeted for degradation [20]. Co-administration of the proteasome inhibitor Bortezomib counteracts this effect. However one could argue that targeting the apoptotic pathway downstream by facilitating a more direct activation of Bax/Bak might prove more efficacious. The main logic for BH3 mimetics is that directly abrogating the interaction between pro- and anti-apoptotic proteins holds more promise than anti-tumor drugs that act upstream in the pathway [21].

PRO-SURVIVAL BCL-2 FAMILY PROTEINS AND THEIR ROLES IN CHEMORESISTANCE

Bcl-2 and Bcl-xL are closely related proteins. Both molecules are highly overexpressed in many types of cancers [22,23]. Overexpression of Bcl-2 is observed in 30-60% of prostate cancer at diagnosis and in nearly 100% of hormone-refractory prostate cancer [24,25]. The expression level of Bcl-2 protein also correlates with resistance to a wide spectrum of chemotherapeutic agents and radiation therapy [25,26]. Overexpression of Bcl-2 protein decreases the pro-apoptotic response to such cellular insults as irradiation, chemotherapy, and androgen withdrawal, leading to resistance to the treatments [27-31]. Bcl-xL is found to be overexpressed in almost 100% of hormone-refractory prostate cancer and is associated with advanced disease, poor prognosis and shortened survival, and significantly associated with recurrence and metastasis [32-34]. Interestingly, animal studies have also shown that Bcl-2 and Bcl-xL are highly up-regulated after castration [35,36]. Clearly, the transition of prostate cancer from androgen-dependence to androgen-independence is accompanied by a number of genetic and epigenetic changes, but overexpression of the key survival proteins Bcl-2 and Bcl-xL presents a major obstacle to chemotherapeutic induction of apoptosis [22,26,37,38].

Until recently, myeloid cell leukemia-1 (Mcl-1) was a lesser studied anti-apoptotic member of the Bcl-2 family. However, studies have shown it to be a highly regulated molecule that promotes cell viability and can contribute to human malignancy when dysregulated [39-42]. The expression of Bcl-2, Bcl-xL and Mcl-1 increases during progression of prostate cancer, a finding relevant to the hormone-insensitive, metastatic phenotype of most advanced adeno-carcinoma of the prostate [26]. Mcl-1 was expressed in 52 of 64 (81%) tumors, compared with only 9 of 24 (38%) cases of precancerous prostatic intraepithelial neoplasia

(PIN) ($P < 0.001$) [26]. In addition, the percentage of Mcl-1-positive cells was typically higher in Gleason grade 8 to 10 tumors and metastases than in PIN or lower grade tumors ($P = 0.025$) [26]. Another model of late stage prostate adeno-carcinoma highlighted the anti-apoptotic role of Mcl-1 overexpression that was driven by the inflammatory cytokine IL-6 [43]. The finding that Mcl-1 can add to the anti-apoptotic affect of Bcl-2 and Bcl-xL overexpression has become a major consideration when designing strategies to overcome chemoresistance.

BCL-2 AND BCL-XL ARE PROMISING TARGETS FOR OVERCOMING RESISTANCE OF AI PROSTATE CANCER

Since Bcl-2 and Bcl-xL potently inhibit apoptosis induced by a wide variety of stimuli, their overexpression has been associated with progression to androgen-independence in prostate cancer, reflecting the consequence of blocking the programmed cell death that would normally ensue upon androgen deprivation in prostate epithelial cells [4,23,44-46]. Since most of the current chemotherapeutic agents for prostate cancer work by indirectly inducing apoptosis in cancer cells, high levels of Bcl-2 and Bcl-xL protect cancer cells from chemotherapy-induced apoptosis, thus making cancer cells resistant to chemotherapy [27,30,31,47,48]. Indeed, the expression levels of Bcl-2 and Bcl-xL proteins correlate with resistance to a wide spectrum of chemotherapeutic drugs and radiation therapy [13,23,29,31]. Therefore, overexpression of Bcl-2 and Bcl-xL likely play a critical role in the failure of current chemotherapy for the treatment of hormone-refractory prostate cancer.

Since several lines of evidence strongly suggest that Bcl-2 and Bcl-xL play a key role in the progression to AI and resistance of AI disease to chemotherapy in prostate cancer [2,26,29,37,49-51], limiting their expression and/or activity has become one focus for molecularly targeted therapy (Fig. (1)). Anti-sense Bcl-2 and Bcl-xL studies provided the first evidence that repressing Bcl-2 and Bcl-xL expression may be an effective new therapeutic strategy for the treatment of advanced prostate cancer [6,33,36,37,52-54]. Both anti-sense Bcl-2 and anti-sense Bcl-xL have been shown to delay the progression to AI in animal models [36,53]. While the combination of paclitaxel with either anti-sense Bcl-2 or anti-sense Bcl-xL increases the chemosensitivity of prostate cancer and delays the progression to androgen independence, including both anti-sense molecules as co-therapy achieves a much greater effect in delaying the this transition [36].

ANTI-SENSE THERAPY FOR OVERCOMING CHEMORESISTANCE IN AI PROSTATE CANCER

Whether or not suppressing anti-apoptotic protein expression in AI Prostate Cancer by targeting the mRNA with anti-sense oligonucleotides could be a useful therapeutic strategy remains an open question. Before the advent of siRNA modalities, many proof-of-concept experiments in the field of chemosensitization utilized anti-sense approaches to show that knocking down a specific protein could affect the treatment outcome. Several studies have shown that Bcl-2 anti-sense therapy sensitizes cancer cells to chemo-drugs *in vitro* and *in vivo* in various tumor models including prostate cancer with high levels of Bcl-2 and has a synergetic effect in combination with chemotherapy [6,33,48,52,54-60]. In our own studies in the MDA-MB-231 xenograft model of breast cancer, while Bcl-2 anti-sense alone only inhibited tumor growth, a complete tumor regression was seen when combined with a low dose taxotere. Importantly, after the treatment was stopped, mice remained tumor free for more than 6 months. These results are consistent with the anti-apoptotic function of Bcl-2. Genta Incorporated developed an anti-sense Bcl-2 therapy (Genasense) that has shown promise in treating certain malignancies [61]. However, a multicenter trial testing

Genasense/docetaxel vs. docetaxel alone to treat advanced prostate cancer did not reveal any advantage to the combinatorial approach [62]. This does not limit the utility of Genasense administration to treat tumors where Bcl-2 is the major pro-survival protein. Currently, several advanced clinical trials are ongoing which compare various chemotherapeutic agents with or without Genasense for treating both solid and non-solid tumors of multiple origins (reviewed extensively in [61]). Earlier clinical trials of Bcl-2 anti-sense from Genta in humans have demonstrated clinical activity as a single agent with mild toxicities. Notwithstanding these observations as well as use in advanced CLL treatment [63], extensive clinical evaluations using Bcl-2-specific anti-sense have resulted in an overall disappointing experience [61].

A major drawback of the anti-sense approach for targeting Bcl-2 is the fact that Bcl-xL and Mcl-1 levels are also often increased in tumors. Multiple *in vitro* and *in vivo* studies have shown that both Bcl-xL and Mcl-1 can potently inhibit apoptosis. Not surprisingly, down-regulating Mcl-1 by either anti-sense oligonucleotides or siRNA resulted in increased sensitivity to radiation and chemotherapy in various cancers [64-70]. Perhaps a mixture of anti-sense molecules with a broader spectrum of Bcl-2 family inhibition might increase the efficacy of this approach. However, based on the questionable utility of single agent anti-sense for AI prostate cancer mentioned earlier, other therapeutic alternatives may prove more efficacious.

RNAI-BASED THERAPEUTICS TARGETING THE BCL-2 FAMILY PROTEINS

siRNA and shRNA

The advent of RNA interference to degrade specific mRNA molecules and exact a *de facto* silencing of their prospective protein progeny has revolutionized biological inquiry in countless ways. Targeting overexpressed molecules that play a role in malignancy has been a logical step in the application of this technology. The problem that has arisen is one of delivery. The simplest avenue toward RNA interference is to transfect or administer small, double – stranded and hence complementary siRNA molecules directly into cells or subjects, respectively. By using this strategy to target Bcl-2 mRNA, investigators found that Bcl-2 protein levels, cell viability, and proliferation rate were significantly reduced in a PC-3 cell model of prostate cancer [71]. Similar results were seen in another model of late stage prostate cancer by targeting Mcl-1 in LnCAP-IL-6+ cells [43].

Expression vector based expression of shRNA, which is processed by endogenous machinery into siRNA, is an alternate strategy to elicit RNA interference. Studies in prostate cancer cell lines as well as others have demonstrated the efficacy of this strategy in downregulating Bcl-2 family proteins and sensitizing cells to apoptosis [72,73]. For example, downregulation of Bcl-2 by shRNA increased sensitivity to UV induced apoptosis of AI prostate cancer cells *in vivo* [74]. Recently, several groups have developed constructs and/or systems to silence multiple gene products thought to cooperate in oncogenesis. Cheng and colleagues created an shRNA coexpression plasmid that efficiently and simultaneously silences *Bcl-2*, *Survivin*, *Akt1*, *Erk2*, *CyclinE* and *NFκB* [75]. They found that knocking down only Bcl-2 and Survivin had a similar growth inhibitory effect as silencing all six, but better inhibition than Bcl-2 alone. The chemosensitization potential of this approach was not determined, however PC-3 prostate cancer cells were sensitized to apoptosis upon plasmid delivery. This multi-factorial knock-down system may have great potential for adjuvant therapy in malignancies where the molecular targets of oncogenesis are known.

microRNA

A veritable explosion of evidence has recently emerged regarding the link between microRNA (miRNA) expression and cancer progression. miRNAs regulate a variety of

biological processes, including developmental timing, signal transduction, tissue differentiation and maintenance, and carcinogenesis. These small non-coding RNAs control gene expression post-transcriptionally by binding short sequences on mRNA molecules and either inhibiting translation or targeting the molecule for degradation [76,77]. Disruption of miRNA expression levels in tumor cells may result from distorted epigenetic regulation of miRNA expression, abnormalities in miRNA processing genes or proteins, and/or the location of miRNAs at cancer-associated genomic regions [78].

Ittman and colleagues initiated a comprehensive microarray-based investigation into the role of miRNAs in prostate cancer [79]. Using probes against 328 known and 152 prospective miRNA targets, they compared miRNA levels in benign peripheral zone prostate tissue vs. prostate cancer samples from radical prostatectomies. Interestingly, they observed a global downregulation of all sampled miRNAs in the malignant samples. They found that miR-16 and miR-29a levels, which control Bcl-2 and Mcl-1 levels respectively, were decreased in malignant tissue vs. benign control samples. This study confirmed previous reports that miR-16-1, as well as miR-15a, play important roles in Bcl-2 family regulation (recently reviewed by Aqeilan *et al.* [80]).

The Bcl-2 protein is also directly regulated by miR34 and loss of this regulatory molecule has been linked to chemoresistance. miR-34 has been shown to be a direct target of p53 and a potential tumor suppressor. Over 50% of human cancers have p53 loss of function and miR-34 expression correlates with p53. Our group has shown that transfecting pancreatic cancer cell lines with miR-34 downregulates pro-survival proteins, including Bcl-2, as well as gene products implicated in self-renewal [81]. This effect leads to increased chemosensitivity, inhibited tumor initiation, as well as decreased tumor sphere formation. These data in a pancreatic carcinoma model together with the knowledge that Bcl-2 and Mcl-1 regulation is suppressed in prostate cancer make miRNAs 15a, 16-1, 29a and 34 attractive candidates for molecularly targeted therapy.

Although promising results have been seen in model systems, the clinical challenge in human patients regarding miRNA delivery remains daunting. Lessons may be learned by the approaches taken to overcome the problem of targeting siRNA. The painstaking research involved in addressing this obstacle was recently reviewed by Gondi and Rao [82]. The authors proposed that the most effective strategies would most likely involve nanoparticle delivery of protected RNA molecules or plasmids that would express the appropriate RNAs or precursors. Indeed, Calandro Pharmaceuticals has begun a clinical trial of CALAA-01, whose active ingredient is a tumor-targeted and nanoparticle stabilized (to inhibit nuclease degradation) siRNA to target ribonucleotide reductase. Another review highlighted recent successes in nanoparticle delivery of lipids as well as the challenges facing RNA delivery in humans [83]. In addition to lipid-protein complexes, protecting RNA with either protein or carbohydrate moieties is showing promise. The potential for siRNA as a therapeutic is not limited to oncology. Several companies have initiated siRNA based clinical trials for other diseases. The progress of nanoparticle delivery systems will hopefully lead to a new era in cancer treatment and miRNAs, siRNA and vector based approaches for their expression are prospective cargo.

SMALL-MOLECULE BH3 MIMETICS FOR TREATING AIPROSTATE CANCER

Non-peptide, small molecule inhibitors of Bcl-2 and Bcl-xL have considerable therapeutic advantages over anti-sense, antibody, and peptide approaches, including better oral availability, better stability and/or low cost [84,85]. The anti-apoptotic function of Bcl-2/Bcl-xL is attributed, at least in part, to their ability to heterodimerize with pro-apoptotic

members such as Bak, Bax and Bad [45,84,86]. The experimental structures of Bcl-2 and Bcl-xL show that BH1 (Bcl-2 homology domain 1), BH2 and BH3 domains of Bcl-2 and Bcl-xL form a hydrophobic binding pocket (the BH3 binding pocket) into which Bak or Bad BH3 domain binds [87,88]. This binding pocket in Bcl-2/Bcl-xL is essential for its anti-apoptotic function. It has been shown that small molecules that bind to this BH3 binding site in Bcl-2/Bcl-xL are capable of blocking the hetero-dimerization of Bcl-2/Bcl-xL with pro-apoptotic members in the Bcl-2 protein family, such as with Bim, Bad, Bak and Bax. This, in turn, inhibits the anti-apoptotic function of Bcl-2/ Bcl-xL and induces apoptosis in cancer cells with Bcl-2/Bcl-xL overexpression [45,85,89]. The clinical progress of several non-peptide, small molecule inhibitors of Bcl-2, Bcl-xL and Mcl-1 are highlighted here (Table 1).

One exciting compound now entering Phase I clinical trials is the highly potent inhibitor of Bcl-2/xL, ABT-263. Recent studies [90-94] show that cancer cells resistant to the parent compound ABT-737 have high levels of Mcl-1, and knock-down of Mcl-1 promotes ABT-737-induced apoptosis. A modification of ABT-737 produced ABT-263 which exhibits significantly enhanced binding to Bcl-2 that is in fact several orders of magnitude tighter than ABT-737. Oral availability of this compound is also better. However both of these compounds suffer from an inability to bind Mcl-1. Since many cancer cells have high levels of Mcl-1, they are intrinsically resistant to ABT-263 or other specific Bcl-2 inhibitors. As detailed in a review by Chonghaile and Letai, any non-pan-Bcl-2 inhibitor could potentially spur selection for other pro-survival family members and negate the therapeutic approach [17]. Hence, the focus of the field has moved towards developing a broad spectrum Bcl-2 family inhibitor.

A naturally occurring small molecule inhibitor of anti-apoptotic Bcl-2 family members has shown promise in overcoming chemo/radioresistance in various tumor models including prostate cancer [95,96]. (-)-Gossypol, a natural product from cottonseed, has been identified as a BH3-mimetic small molecule inhibitor of Bcl-2/Bcl-xL/Mcl-1, or so-called pan Bcl-2 inhibitor, and induces apoptosis in various types of cancer [97-102]. We previously reported that (-)-gossypol induces apoptosis by blocking the interaction of Bcl-xL with Bax and Bad in PC-3 cells [103]. We have also shown that (-)-gossypol sensitized androgen-independent prostate cancer cells to radiation and chemotherapy both *in vitro* and *in vivo* [103,104]. (-)-Gossypol is now in Phase II-III clinical trials for hormone-refractory prostate cancer and other types of cancer at multiple centers in the United States (AT-101, <http://ClinicalTrials.gov>). A summary of findings from early stage clinical trials of AT-101 and other BH3 mimetics has been reviewed elsewhere [61]. To date, promising results have been seen using these agents alone, as well as in combination with existing anticancer therapeutics. For advanced prostate cancer, combinatorial trials with chemotherapeutics such as docetaxel are ongoing, but in a preliminary report, a 24% overall response rate was seen with docetaxel, AT-101 and prednisone [61].

POTENTIAL FOR BCL-2 CONVERTING PEPTIDES AS MOLECULARLY TARGETED THERAPY

Recently, elegant work by Zhang and colleagues uncovered an intriguing function for a newly discovered Bcl-2 binding protein [105,106]. An orphan nuclear receptor, Nur77, binds a loop of region of Bcl-2 between its BH3 and BH4 domains. This binding catalyzes a profound conformational change in Bcl-2 which unmasks its BH3 domain. The minimal peptide of Nur77 responsible for this conversion was dubbed the Nur77-derived Bcl-2-converting peptide or NuBCP. Binding of this peptide induces a functional switch in Bcl-2 from a pro-survival to a pro-apoptotic molecule via three empirically deduced mechanisms: loss of Bax/Bak binding capability, loss of BH3 only protein binding, and/or acquired ability

to bind Bcl-xL and prevent its pro-survival function. Whether or not the Bcl-2 BH3 domain could also inhibit Mcl-1 function is yet to be determined. If developed into therapeutics, Bcl-2 converting peptides or mimetics of these molecules could exact a marked inhibition of pro-survival Bcl-2 family members.

BESIDES APOPTOSIS, AUTOPHAGY CAN ALSO BE INDUCED BY BCL-2 INHIBITION

Although this review mainly focuses on how derepressing apoptosis by Bcl-2 inhibition can overcome chemoresistance, autophagy has received increasing attention as an alternate mechanism of action for the BH3 mimetic ABT-737. The more recently recognized BH domain containing protein, Beclin-1, has been shown to play a critical role in autophagic death of cancer cells exhibiting high levels of Bcl-2. Bcl-xL and Bcl-2 are not only anti-apoptotic but also anti-autophagic proteins, mainly residing on the mitochondrial and endoplasmic reticulum membranes. The pro-survival role of Bcl-2/xL at the level of the mitochondria is well-established, but their binding to Beclin-1 on the ER can protect the cell from autophagic death. In mitochondria, Bcl-2 and Bcl-xL bind to Bad, Bak or Bax. When ABT-737 binds to Bcl-2/Bcl-xL [107], Bax and/or Bak are released from inhibition and oligomerize to induce apoptosis. ABT-737 binds to Bcl-2/xL and induces apoptosis by blocking the interaction between Bcl-2 and its pro-apoptotic targets. It can also induce autophagy through modulating the interaction between Bcl-xL and Beclin1 [108-111]. Another BH3-mimetic, Obatoclax (GX15-070), may also induce autophagic death under certain conditions and is in clinical trials now [61,95].

CONCLUSIONS AND FUTURE PERSPECTIVES

Tailoring cancer treatment to address the specific molecules responsible for tumor growth and progression is a promising new area of clinical oncology. In fact, a major goal of the NCI is to push toward determining the genome/proteome of each patient's cancer and design a molecularly targeted strategy for treatment. For many years, targeting molecules that would stimulate apoptosis has been a main focus, since inhibition of this pathway is often a factor in malignant cell survival. Now that many of the major players have been identified, the field is moving toward attacking the pathway at every level. In one of the studies highlighted here, not only Bcl-2, but Survivin and Akt1, were concomitantly silenced along with other pro-survival genes. This approach represents an exciting strategy for treating patients with multi-factorial disease. A tremendous amount of effort has given us a repertoire of verified siRNA, shRNA and miRNA sequence information from which to choose when testing for efficacy in our ever-expanding model systems of carcinogenesis.

Besides apoptosis, there may be an additional pathway worth targeting when considering the best way to kill androgen independent prostate cancer cells as well as other cancers. Recently, autophagy has emerged as a target pathway and promising breakthroughs have been made in killing cancer cells. Concomitant stimulation of apoptosis and autophagy with ABT-737 and rapamycin, respectively, along with IR gave promising results in a NSCLC model [108]. Our group recently reported upregulation of Noxa and Puma as a novel molecular mechanism of action of (-)-gossypol in high Bcl-2 AI prostate cancer cells. This induction of pro-apoptotic BH3 only proteins is seen with many chemotherapeutics, but (-)-gossypol effects a second hit by inhibiting the action of pro-survival proteins Bcl-2/Bcl-xL and Mcl-1. As others have reported, the interplay between apoptosis and autophagy is fundamental to discerning how BH3 mimetics induce cell death. Clearly, understanding the proteome of each patient's cancer will help define the treatment regimen with the greatest chance of success.

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Fig. (1). Multiple modes of Bcl-2 family inhibition as molecularly targeted therapy

Two empirically supported schemes of Bcl-2/xL/Mcl-1 mediated inhibition of apoptosis are represented. In Scheme 1, Bcl-2/xL/Mcl-1 directly inhibit Bax/Bak homo-oligomerization by binding the Bax/Bak BH3 domain. In Scheme 2, Bcl-2/xL/Mcl-1 sequester BH3 only protein activators of Bax/Bak such as Bim. Without Bim mediated activation, Bax/Bak fail to homo-oligomerize and induce apoptosis. In either scenario, BH3 mimetics, RNAi, or Bcl-2 converters counter-act the pro-survival activity of Bcl-2 overexpression by either inhibiting the interactions of Bcl-2/xL/Mcl-1 with pro-apoptotic (Bax/Bak or Bim) proteins or reducing the level of Bcl-2. The proposed mechanism for how NuBCP induces a conformational change in Bcl-2 that exposes its BH3 domain and converts Bcl-2 from a pro-survival to a pro-apoptotic protein is illustrated.

Table 1

Select Clinical Trials Targeting the Bcl-2 Family Proteins

Investigational Drug (Company)	Co-therapy	Cancer type	Phase	ClinicalTrials.gov Identifier
ABT-263 (Abbot)	none	multiple	I	NCT00743028
	none	multiple	I	NCT00982566
	gemcitabine	solid tumor	I	NCT00887757
	docetaxel	solid tumor	I	NCT00888108
AT-101 (Ascenta Therapeutics)	docetaxel + prednisone	prostate	I/II	NCT00286793
	docetaxel + prednisone	HRPC	II	NCT00571675
	none	HRPC	I/II	NCT00286806
	none	B-cell lymphoma	II	NCT00275431
GX15-070 (Gemix X)	none	CLL	I	NCT00600964
	docetaxel	lung	I/II	NCT00405951
Oblimersen (Genta Inc.)	docetaxel	prostate	II	NCT00085228

HRPC = hormone refractory prostate cancer, CLL = chronic lymphocytic leukemia, AT-101 = (-)-gossypol, GX15-070 = obatoclax