Minireview

Tumor Chemosensitization Strategies Based on Apoptosis Manipulations

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Background

MDR is a major obstacle to the effective treatment of cancer. Drug resistant cancers are either inherently untreatable (intrinsic resistance) or they have progressed to develop resistance to a wide variety of anticancer agents over the course of treatment (acquired resistance). The term MDR is used to describe the ability of tumor cells exposed to a single cytotoxic agent to develop resistance to a broad range of structurally and functionally unrelated drugs. Numerous mechanisms are known to contribute to this phenomenon, including overexpression of drug efflux pumps, increased activity of DNA repair mechanisms, altered drug target enzymes, and overexpression of enzymes involved in drug detoxification and elimination. Because most chemotherapy approaches ultimately elicit their effects via apoptosis, alterations at the level of apoptosis control provide yet another mechanism by which drug resistance may occur. This review will focus on some of the strategies that have been used in an attempt to chemo sensitize resistant tumors by manipulating dysregulated apoptosis pathways.

Mechanisms of Drug Resistance Mediated by Alterations in Apoptosis Pathways

The decision as to whether a cell undergoes apoptosis or continues to progress through the cell cycle is dependent on the interplay of a complex set of genes and proteins that interact to regulate cell cycle progression. Drug resistance can emerge if cells alter the expression of proteins that regulate the propagation of signals arising from cellular insults, such as chemotherapy, to protect against apoptosis. Although many details of the apoptotic pathway are still not completely understood, several proteins are known to be important regulators of this process.

p53 Tumor Suppressor

The p53 tumor suppressor plays a significant role in ensuring genetic stability, and several studies have found that lack of p53 function greatly increases the risk of malignancy and is correlated with aggressive tumor growth and recurrence. Its importance in cancer is highlighted by estimations that it is mutated in >50% of all human tumors. Numerous cellular insults result in p53 accumulation, which induces cell cycle arrest in G1, or triggers apoptosis, depending on the extent of DNA damage. The integrity of this cellular defense mechanism is crucial for the maintenance of an intact genome, and, therefore, p53 is often referred to as the “guardian of the genome.” Consequently, loss of p53 function, either through direct mutation of the p53 gene itself or via regulators of p53 function, results in the removal of this important cell cycle checkpoint, and downstream activation of pro-apoptotic factors is no longer initiated. Consequently, cells are able to proliferate even in the presence of cellular insults and drug resistance develops.

Bcl-2 Family Proteins

Apoptosis is regulated in part by the Bcl-2 family of proteins whose members include pro-apoptotic Bax and Bak and antiapoptotic Bcl-2, Bcl-xL, and Mcl-1. The relative ratio of these proteins determines the sensitivity or resistance of cells to various apoptotic stimuli. Overexpression of antiapoptotic proteins or down-regulation of pro-apoptotic proteins blocks the release of cytochrome c from mitochondria, which is an early event of apoptosis that precedes caspase and endonuclease activation. Bax and Bak proteins are believed to translocate from the cytoplasm to the outer mitochondrial membrane where they oligomerize to form pores through which cytochrome c is released. These effects are antagonized by up-regulation of antiapoptotic Bcl-2 family members, which inhibit oligomerization and diminishes cytochrome c release, thereby blocking apoptosis. This has been correlated with decreased sensitivity to a wide range of chemotherapeutic agents and the emergence of resistance (1).

IAP Family Proteins

IAPs are a family of apoptosis suppressor proteins that were first discovered in baculoviruses. Although the precise mechanisms by which IAPs block cell death have not been fully elucidated, they are believed to act as caspase inhibitors. Caspases are important proteins involved in the proteolytic cleavage of key cellular components, such as proteins in-
volved in DNA repair pathways, and cytoskeletal and structural proteins, such as lamin B1 and actin. These processes ultimately disassemble the cell and give rise to many of the biochemical and morphological hallmarks of apoptosis. Because the binding of IAPs to caspases blocks caspase function, the relative ratios of caspases and IAPs likely determines whether a cell undergoes apoptosis. Alterations in this ratio in favor of IAP binding can therefore contribute to MDR. The IAP family member XIAP specifically binds to the effector caspase-9 and prevents it from initiating downstream apoptotic events, such as activation of pro-caspase-3. Although the mechanism of action of the IAP family member survivin is less clear, both proteins have been shown to be highly expressed in various malignant tissues, and their expression has been correlated with poor clinical outcome and resistance to chemotherapy drugs and ionizing radiation (2).

**Sphingolipid Family Lipids**

The sphingolipid breakdown product ceramide has recently become the subject of considerable interest as a mediator of apoptosis and coordinator of cellular responses to stress. Intracellular ceramide levels increase after exposure to many chemotherapy drugs, ionizing radiation, and cytokines, and cells subsequently exhibit morphologies typical of apoptosis. Ceramide presumably exerts its effects through direct molecular interactions with specific target molecules that, in turn, activate subsequent signaling cascades. However, the exact nature of these targets and details of the downstream signaling events are not fully understood. Some of the direct targets that have been identified include ceramide activated protein phosphatase and kinase, both of which promote growth suppression and initiate apoptosis. Endosomal/lysosomal cathepsin D has also been identified as a ceramide-binding protein, and it is believed that ceramide produced in this compartment induces release of the protease into the cytosol, where it initiates a proteolytic cascade leading to apoptosis (3). More recent studies are also pointing toward cytosol, where it initiates a proteolytic cascade leading to this compartment induces release of the protease into the mitochondrial membranes (4). Therefore, loss of ceramide production or increases in its metabolism serve to decrease apoptosis signaling.

A key example of altered ceramide metabolism involves accumulation of its noncytotoxic GlcCer metabolite. Several drug resistant cancer cell lines have been shown to express higher levels of GlcCer than their drug sensitive counterparts, and analysis of clinical tumor specimens from patients who failed conventional chemotherapy revealed higher GlcCer levels than samples taken from those patients who responded well to treatment (5). The level of activity of the GCS enzyme, which converts ceramide to GlcCer, is believed to be responsible for this aspect of the MDR phenotype, as was demonstrated by retroviral transfection of GCS into drug sensitive MCF7 human breast cancer cells (6).

Other sphingolipids, in particular the ceramide metabolites sphingosine and sphingosine-1-phosphate, are also regulators of cell death and survival. S1P tends to oppose the pro-apoptotic effects of ceramide and sphingosine and has been implicated in cell survival and proliferation (7). Consequently, up-regulation of the sphingosine kinase enzyme responsible for its production may contribute to MDR by protecting the cell from apoptosis.

**Other Cell Signaling Pathways**

In addition to targeting specific proteins involved in initiating the apoptotic machinery, modulating the cell signaling pathways that control cellular proliferative responses may provide an alternate strategy for inducing cell death and increasing susceptibility to apoptosis. Cell signaling mediated by protein kinases, phosphatases, and transcription factors are all potential therapeutic targets suitable for this approach (reviewed in Ref. 8), e.g., the apoptosis-inducing calcium-activated phosphatase calcineurin acts in part by dephosphorylating Bad, thereby promoting heterodimerization with Bcl-xL to initiate apoptosis. Calcineurin agonists may therefore be used to aid in sensitizing resistant tumors known to overexpress antiapoptotic Bcl-xL. The phytochemical resveratrol is being investigated as a chemoprotective agent and inducer of apoptosis that acts by inhibiting nuclear factor κB-mediated transcription of antiapoptotic Bcl-2 and IAP family members. Survival signaling via kinases of the PI3K/Akt pathway is another potential target. Increased activity of Akt and PI3K and mutations in PTEN, its negative regulator, are associated with malignancy and render cells insensitive to apoptosis induction. Examples of chemosensitization strategies that act by altering such signaling targets are discussed in the sections to follow.

**Strategies to Cemosensitize Resistant Tumors on the Basis of Modulating Apoptosis Pathways**

Despite vast improvements in our understanding of the mechanisms of drug resistance, the ability to modulate MDR has been complicated by the fact that many human tumors simultaneously exhibit multiple resistance mechanisms. Although MDR is a multifactorial phenomenon, a lack of cellular apoptosis is the ultimate result regardless of the mechanism(s) involved. An increased understanding of the proteins and pathways involved in apoptosis signaling, combined with evidence that many anticancer agents induce their cytotoxic effects via apoptosis, has spawned great interest in developing approaches to chemosensitize tumors by altering apoptosis regulation. The work described in the previous sections provided a background for research efforts directed at chemosensitizing resistant tumors by targeting the protein families described above. Some recent developments in this field are reviewed below and have been summarized in Table 1 and Figs. 1 and 2.

**Inhibition of Pro-Survival Regulators**

**Antisense Down-Regulation of Bcl-2 Family Proteins.**

Given that antiapoptotic Bcl-2 family member proteins are overexpressed in many types of cancer, numerous studies...
have investigated the effect of antisense therapies directed against these targets on drug sensitivity, and several examples of true chemosensitization have been documented. Phosphorothioate-based Bcl-2 antisense has been the most extensively studied treatment to date in this regard. Preclinical studies in mice bearing Bcl-2-overexpressing human B-cell lymphoma showed complete cures in all mice after treatment with Bcl-2 antisense + low-dose cyclophosphamide (9), and infusion of Bcl-2 antisense into mice with human melanoma xenografts showed markedly reduced tumor growth (10). Several clinical trials with Genasense (Bcl-2 antisense; Genta, Berkeley Heights, NJ) are currently underway to investigate its effectiveness in combination with anticancer drugs for the treatment of a variety of neoplasms. Phase I clinical data in patients with relapsed and refractory lymphoma showed decreased Bcl-2 protein levels with evidence of antitumor activity (11), and a Phase I study in patients with relapsed non-Hodgkin’s lymphoma showed one complete response, two partial responses, and nine cases of stable disease (12). A Phase II/I trial of Genasense in combination with paclitaxel in refractory small cell lung cancer showed disease stabilization, although no objective responses were observed (13). Perhaps the most encouraging evidence of therapeutic activity was observed in a Phase I/I study combining Genasense with dacarbazine, where several antitumor responses in patients with resistant malignant melanoma were observed (14). Phase II trials are currently underway for acute myeloid leukemia, chronic lymphocytic leukemia, mantle cell lymphoma, and prostate cancer, and Phase III trials are ongoing in chronic lymphocytic leukemia, multiple myeloma, non-small cell lung cancer, and malignant melanoma, for which Genasense has been awarded Fast Track status.

Although still in preclinical testing, antisense down-regulation of Bcl-xL sensitized glioblastoma cells to paclitaxel (15), and synergy was observed with dexamethasone in leukemia cells (16). Interestingly, a bispecific antisense molecule that targets both Bcl-2 and Bcl-xL was demonstrated to induce apoptosis in cells derived from resistant human glioblastoma tumors (17). It will be interesting to see if this agent shows more activity over antisense directed against Bcl-2 or Bcl-xL alone when evaluated in animal models and clinical trials.

**Antisense Down-Regulation of IAP Family Proteins.** A similar approach to the Bcl-2 family proteins is being taken to down-regulate IAP family members using antisense gene and oligonucleotide therapy strategies. Although still in the preclinical stages of development, in vitro and in vivo studies targeting survivin with antisense oligonucleotides have been shown to induce apoptosis, reduce tumor growth potential, and sensitize tumor cells to Taxol, cisplatin, etoposide, γ-irradiation, and immunotherapy (18). Down-regulation of survivin levels by antisurvivin antisense corresponded to complete chemosensitization of acute lymphoblastic leukemia cells to doxorubicin in vitro (19). An in vivo study demonstrated complete eradication of tumors derived from mouse thymic lymphoid tumors in response to survivin antisense single therapy (20). In a similar manner, down-regulation of XIAP has been shown to induce apoptosis in chemoresistant ovarian cancer cells (21). XIAP antisense decreased protein expression and increased apoptosis in a dose-dependent manner in four MDR bladder cancer cell lines, and combination treatment with doxorubicin enhanced cytotoxicity (22). Another preclinical study demonstrated activation of caspase-3 and poly ADP ribose polymerase in response to antisense oligonucleotides targeting XIAP, which enhanced the therapeutic activity of vinorelbine in

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**Table 1**  
A summary of apoptosis pathways illustrating some chemosensitization targets and approaches being used for the sensitization of resistant tumors. FTI, farnesyl transferase inhibitor; DAG, diacylglycerol; PKB, protein kinase B; PARP, poly ADP ribopolymerase.

<table>
<thead>
<tr>
<th>Drug/Agent</th>
<th>Company</th>
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<tbody>
<tr>
<td>Genasense</td>
<td>Genta</td>
<td>Bcl-2 antisense</td>
<td>Phase II/III</td>
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<td>Safingol NCI</td>
<td>PKC inhibitor</td>
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<td>NCI</td>
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<td>ELL-12 NCI</td>
<td>Farnesyl transferase</td>
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<td>Cdk/PKB</td>
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<td>Flavopiridol</td>
<td>Cdk/PKB</td>
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<tr>
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<td>SCH/66336</td>
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<td>Cdk/PKB</td>
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**Fig. 1.** Chemosensitization strategies based on manipulating enzymes involved in sphingolipid metabolism. GlcCer, glucosylceramide; GCS, glucosylceramide synthase; PDMP, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; SM, sphingomyelin; CDase, ceramidase; NOE, N-oleylethanolamine; SK, sphingosine kinase; DMS, dimethylsphingosine.

**Fig. 2.** A summary of apoptosis pathways illustrating some chemosensitization targets and approaches being used for the sensitization of resistant tumors. FTI, farnesyl transferase inhibitor; DAG, diacylglycerol; PKB, protein kinase B; PARP, poly ADP ribopolymerase.
human lung cancer cells (23). Aegera Therapeutics, Inc. is currently moving XIAP antisense toward clinical trials. It will be interesting to see if promising in vitro and preclinical results can be translated into clinical activity.

**Modulating Lipid Mediators of Apoptosis**

**GlcCer Synthase Inhibitors.** The identification of GCS activity and GlcCer levels as markers for drug resistant tumors has provided a new avenue for therapies directed at overcoming MDR. A number of studies has indicated that inhibition of GlcCer synthesis results in MDR circumvention. Liu et al. (24) demonstrated that down-regulation of GCS activity in doxorubicin-resistant MCF7/AdR cells by transfection with GCS antisense resulted in a 30% reduction in GCS activity, which was correlated with a 28-fold increase in doxorubicin sensitivity. Other approaches have used specific GCS inhibitors in combination with conventional chemotherapy drugs. Chemosensitization was observed after treatment with the GCS inhibitor 1-phenyl-2-decanoylamino-3-morpholino-1-propanol plus the tubulin binding drugs paclitaxel and vincristine in neuroblastoma (25) and breast cancer cells (26). Additional evidence of chemosensitization using modulators of ceramide metabolism was demonstrated by Litvak et al. (27), who observed a 3-fold increase in cell death of resistant colon cancer cells by combining irinotecan with the GCS inhibitor 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol and safingol.

**Other Modulators of Sphingolipid Metabolism.** Given the pro-apoptotic nature of ceramide and sphingosine, other strategies to increase intracellular levels of these bioactive lipids have been explored. Pharmacologic suppression of acid ceramidase by N-oleylethanolamine restored ceramide accumulation and sensitivity to cytokine-induced apoptosis in fibroblasts (28) and was demonstrated to increase endogenous ceramide levels and trigger apoptosis in resistant, metastatic colon cancer cells (29). Increases in intracellular sphingosine, either by exogenous sphingosine or treatment with the sphingosine kinase inhibitor dimethylsphingosine, induced apoptosis and sensitized androgen-independent LNCaP cells to γ-irradiation (30). These effects have also been associated with elevated ceramide levels (31). Shirahama et al. (32) demonstrated that dimethylsphingosine significantly inhibited the growth of resistant human epidermoid carcinoma tumors in vivo with evidence of increased apoptosis, and treatment of nude mice with di- and tri-methyl sphingosine derivatives showed growth inhibition of various solid tumors (33).

The sphingosine analogue safingol (dihydrosphingosine) has been reported to enhance the accumulation and cytotoxicity of anthracyclines and Vinca alkaloids in both wild-type and multidrug resistant breast cancer cells (34). The pro-apoptotic and MDR modulation effects of safingol have been attributed to inhibition of both protein kinase C and Pgp-mediated drug efflux, which suggests its utility in the treatment of MDR tumors. Safingol was shown to potentiate the effect of mitomycin C in resistant human gastric cancer cells (35), and it improved the chemosensitivity of human tumor xenografts to doxorubicin and cisplatin (36). A pilot Phase I study demonstrated that safingol could be safely administered with doxorubicin without dose-limiting toxicity or adverse pharmacokinetic interactions (37). Safingol is currently in Phase I trials in pediatric patients.

Using another approach, increased intracellular ceramide levels resulting from treatment with the novel synthetic retinoid fenretinide have been shown to inhibit cell proliferation and induce apoptosis in numerous cancer types in vitro, and antitumor activity has been observed in vivo (reviewed in Ref. 38). This activity is attributed, at least in part, to the generation of intracellular ceramide. When combined with other modulators of sphingolipid metabolism, such as 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol, safingol, or tamoxifen, strong synergistic interactions were observed in neuroblastoma, Ewing’s sarcoma, lung, melanoma, breast, prostate, colon, and pancreatic cancer cell lines (39–41). Fenretinide was also demonstrated to potentiate the effects of cisplatin in vivo in an ovarian carcinoma xenograft model (42). Results from a Phase I/II trial indicate that fenretinide is well tolerated, and when combined with tamoxifen, it resulted in improvement or disease stabilization in 12 of 15 women with metastatic breast cancer (43). It is currently in Phase I trials with paclitaxel and cisplatin in patients with advanced solid tumors and Phase II trials for neuroblastoma, ovarian, small cell lung cancer, and squamous cell carcinoma of the head and neck. Fenretinide has also been investigated as a chemopreventative agent for prevention of secondary cancers. Results of a Phase III secondary prevention trial suggest a benefit in preventing second breast malignancies in premenopausal women with early breast cancer, and a protective effect in women with ovarian cancer has also been observed (44).

**Sensitization Using SM.** On the basis of observations that increases in intracellular ceramide promote apoptosis, efforts promote ceramide accumulation by administration of its precursor, SM, have been explored. Coadministration of SM with the anticancer drug 5-fluorouracil resulted in significant tumor growth inhibition in an HT29 human colonic tumor xenograft mouse model (45), and similar antitumor activity was demonstrated after combination therapy of SM with 5-fluorouracil or irinotecan in other colon cancer models (46). These results were attributed to the enhancement of apoptotic cell death by increasing the intracellular pool of SM that is available for conversion to pro-apoptotic ceramide.

**Ether Lipids.** The ether lipids edelfosine, ilmofosine, and miltefosine are metabolically stable synthetic derivatives of naturally occurring lysophosphatidylcholine that possess significant antitumor activity. Their biological effects include reduction of tumor cell invasion in vitro, inhibition of tumor metastases, tumor regression, cellular differentiation, and selective inhibition of tumor cell proliferation in many cancer cell lines (reviewed in Ref. 47) and are equally effective in drug sensitive and MDR tumor cells. Although malignant cells are highly sensitive to the effects of antitumor ether lipids, normal cells remain relatively unaffected, illustrating the potential selective antitumor properties of this class of drugs. This is believed to result from selective differences in the ability of normal versus tumor cells to incorporate ether lipids into cellular membranes (48). They appear to have multiple mechanisms of action, including inhibition of the PI3K-Akt/PKB survival pathway, interaction with protein ki-
nase C, intracellular activation of the Fas/CD95 death receptor, intracellular acidification, promotion of cytokine production, and altered plasma membrane function and lipid synthesis (reviewed in Ref. 49). Synergy has been observed after combination therapy with conventional chemotherapy drugs, such as doxorubicin, cisplatin, vinblastine, and mitomycin C (50) and radiation (51) in vitro.

Despite the encouraging activity of antitumor ether lipids in vitro, studies in mouse, rat, and human tumor xenograft models have provided mixed results. Of the encouraging results, significant tumor growth delay was observed in several human gynecologic tumor models treated with edelfosine (52), and synergistic antitumor activity was observed with imlfofisone plus cisplatin or cyclophosphamide in fibrosarcoma and human lung xenograft models (53). Topical application of mitelofosine in skin metastases of breast cancer showed complete and partial responses in 30% of patients (54), and remission rates approaching 71% were observed for the same treatment strategy in patients with cutaneous lymphoma (55). Edelfosine was shown to be effective as a purging agent in autologous bone marrow transplantation in clinical studies (56). Clinical trials with ether lipids delivered p.o. or i.v. have provided rather disappointing results, as studies have been limited by gastrointestinal and hemolytic toxicities (50). In an attempt to circumvent these limitations, a liposomal formulation of edelfosine (ELL-12) was developed. It was reported to be two to four times more potent than free edelfosine and was associated with 4–8-fold less acute toxicity. ELL-12 was found to be more effective than free edelfosine against leukemia, lung cancer metastases, and melanoma at lower and nontoxic dosing schedules (57), which translated to a 4-fold improvement in the therapeutic index of ELL-12 over free edelfosine. A Phase I trial in patients with refractory solid tumors using a five consecutive day i.v. dosing schedule for 3 weeks was conducted without significant toxicity. A complete remission in one patient with renal cell carcinoma and disease stabilization in a patient with melanoma were reported (58).

**Altering Cell Signaling Pathways**

**Cell Cycle Control Inhibitors.** Cdk-cyclin complexes consisting of catalytic (Cdk) and regulatory (cyclin) subunits regulate cell cycle progression. Endogenous Cdk inhibitors become activated as part of cell cycle checkpoints in response to DNA damage to induce cell cycle arrest (59). Many human cancers are associated with genetic changes in cell cycle control pathways, which result in improper cell proliferation. Numerous pharmacologic inhibitors of Cdns have been investigated in an attempt to induce apoptosis and enhance the cytotoxicity of coadministered therapeutics by controlling cell cycle progression and inducing apoptosis through inhibition of signal transduction pathways, such as protein kinase C, Erk-1, and the epidermal growth factor receptor (60). The inhibitors 7-hydroxystaurosporine (UCN-01) and flavopiridol have been most extensively investigated to date. Both agents have been shown to decrease cellular levels of antiapoptosis proteins, including members of the Bcl-2 and IAP protein families (61), suggesting their potential utility as both single agent therapeutics and sensitizers to conventional chemotherapeutics. UCN-01, an inhibitor of Cdk1 and Cdk2, induced G1 arrest in several tumor cell lines in vitro (62) and enhanced the cytotoxicity of numerous chemotherapeutic agents (63). In vivo studies demonstrated potent antitumor activity in several solid tumor xenografts (64). Phase I/II clinical studies have revealed that clinically relevant plasma concentrations can be achieved with minimal toxicity. A Phase I trial using a 72-h continuous infusion schedule reported a partial response lasting 8 months in a patient with refractory metastatic melanoma, and a patient with refractory lymphoma had no evidence of disease >3 years after treatment (65).

The flavonoid flavopiridol also induces cell cycle arrest by inhibiting Cdk1 and Cdk2. Its antiproliferative and pro-apoptotic effects have been observed in several in vitro models (60), and synergy has been reported with agents such as cisplatin, paclitaxel, topotecan, and doxorubicin (66). Using in vivo models, flavopiridol enhanced the activity of CPT-11 in resistant human colon cancer xenografts (67) and eradicated tumors in leukemia and lymphoma xenografts (68). Although a Phase I clinical trial with flavopiridol administered as a 72-h infusion every 2 weeks at various doses showed dose-limiting gastrointestinal toxicity, a patient with metastatic gastric cancer had a complete response and remained disease free for >48 months after completing therapy (69). In another study, patients with refractory non-Hodgkin’s lymphoma, colon, or renal cancers achieved a reduction in tumor mass and disease stabilization (70). A Phase II study conducted on 35 patients with renal cancer reported two partial responses (71). A study of 16 patients with advanced gastric carcinoma reported no objective responses (72), and did preliminary results in a Phase II study in patients with metastatic colorectal cancer (73). The limited clinical activity of flavopiridol as a single agent has spawned new trials of the agent in combination with standard chemotherapy approaches based on the synergy observed in preclinical models (74, 75).

**Farnesyl Transferase Inhibitors.** Mutations in Ras oncoproteins play an important role in carcinogenesis and are common in many types of cancer to varying degrees ranging from 30% in myeloid leukemia and lung to 90% in adenocarcinomas of the pancreas (76). Cellular activity of the Ras oncoprotein is regulated through the enzyme farnesyl transferase, which is the most critical step in post-translational modification of the Ras protein. Ras is involved in receptor tyrosine kinase signaling crucial for the transmission of mitogenic stimuli, e.g., activated Ras is known to stimulate the Raf-1/mitogen-activated protein/extracellular signal-regulated kinase/extracellular signal-regulated kinase pathway that stimulates cell division (77). Because Ras signaling is associated with the generation of positive proliferative signals that do not favor apoptosis, inhibitors of the farnesyl protein transferase enzyme (farnesyl transferase inhibitors) have been developed as a therapeutic strategy to inhibit Ras farnesylation and abrogate the cell signaling in Ras-transformed cells (78). Three such inhibitors, BMS-214662, SCH66336 (Sarasar), and R115777 (Zarnestra), are some of the most advanced in clinical development as monotherapy and in combination with standard cytotoxic agents. All
agents are cytostatic, and BMS-214662 is cytotoxic. Their effects in many cell culture and xenograft models have been shown to be elicited through induction of apoptosis and cell cycle arrest in G1 or G2-M, although other via antiangiogenic properties mediated by suppression of expression of vascular endothelial growth factor and by interaction with the PI3K/AKT signaling pathway (79). Interestingly, the agent SCH66336 was demonstrated to directly inhibit Pgp, which suggests its utility in the treatment of refractory tumors and was further demonstrated to synergize with Pgp substrates and inhibitors both in vitro and in vivo (80).

Phase I clinical development of BMS-214662 has demonstrated only modest activity in patients with a variety of advanced solid tumors (81, 82). Severe gastrointestinal and liver toxicities after i.v. and p.o. dosing have largely limited systemic exposure, although no significant toxicity was reported in a study combining BMS-214662 plus paclitaxel, in which tumor and serological responses were observed in two of three patients (83). Phase I evaluation of SCH66336 has also demonstrated gastrointestinal and hematological toxicity (84), although disease stabilization and partial responses in a variety of solid tumors have been observed in combination with gemcitabine (85) or paclitaxel (86). A Phase II study of SCH66336 monotherapy in 21 patients with metastatic colorectal cancer refractory to 5-FU and irinotecan did not result in clinical responses, although stable disease was seen in three patients for several months (87). Phase I evaluation of R115777 monotherapy in advanced leukemias showed no toxicity and reported a 29% response rate, including two remissions (88). Combination therapy of R115777 plus 5-FU and leucovorin in a Phase I study of patients with advanced colorectal or pancreatic cancer showed manageable nonhematological toxicity but dose-limiting hematotoxicity (89). Complete and partial responses have been observed in various solid tumors in combination with other conventional chemotherapeutics (reviewed in Ref. 90), notably with doxetaxel in breast cancer patients in which one complete, four partial, and six stable disease responses were observed out of 19 evaluable patients (91). Phase III studies are currently underway.

Future Directions

The past decade has witnessed significant progress in identifying and understanding the various mechanisms involved in the complex phenomenon of MDR. Examples of preclinical and clinical chemosensitization have been achieved through the successful modulation of key biochemical targets known to be involved in mediating drug resistance. The success of these approaches, combined with a growing appreciation for the diversity of MDR mechanisms and the inter-relatedness between signaling pathways, has spawned increased interest in developing new sensitization strategies aimed at manipulating apoptosis regulation. This is a particularly attractive approach given the fact that most chemotherapy strategies ultimately elicit their effects via programmed cell death.

An increased understanding of the molecular basis of apoptosis regulation in normal and tumor cells has facilitated the development of new therapeutic strategies directed against key apoptosis signaling mechanisms that typically become dysregulated in resistant tumors. The strategies described in this study have provided some examples of encouraging preclinical and clinical results using this apoptosis-based approach. However, MDR is indeed a multifaceted phenomenon, and numerous interacting and potentially redundant pathways control apoptosis in cancer cells. Whether or not apoptosis proceeds is based on a delicate balance between several factors, and it is conceivable that modulation of one target may result in alterations in another pathway that could offset shifts in apoptosis regulation induced by targeted treatments. In addition, given the inherent heterogeneity of tumors, it is possible that multiple resistance mechanisms exist within the tumor population. Consequently, it may not be surprising that individual agents directed against specific apoptosis regulators have not resulted in more dramatic improvements in patient responses to chemotherapy.

Taken together, these observations highlight the need for a multifunctional approach to chemosensitization in which multiple pathways and intracellular proteins are simultaneously targeted to make significant in-roads into improving the clinical activity of current and future anticancer agents.

References


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