Review

Caspase Regulation in Non–Small Cell Lung Cancer and its Potential for Therapeutic Exploitation

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ABSTRACT
Metastatic non–small cell lung cancer (NSCLC, stages IIIB/IV) is one of the most common and rapidly lethal causes of cancer related mortality worldwide. Efficacy of chemotherapy, the mainstay of treatment, is limited due to resistance in the vast majority of patients. NSCLC cells exhibit intrinsic apoptosis resistance. Understanding the molecular basis of this phenotype is critical, if therapy is to move beyond the therapeutic plateau that has been reached with conventional chemotherapy. Caspases occupy a pivotal position in the final common pathway of apoptosis. Increasing evidence suggests that these proteases are constitutively inhibited in NSCLC. This review discusses current knowledge relating to caspase regulation in NSCLC and highlights novel strategies for reversing the apoptosis resistant phenotype, with potential to accelerate development of effective therapy.

INTRODUCTION
Lung cancer presents an enormous burden to health systems worldwide. In the United Kingdom; for example, 27% of all cancer death is due to lung cancer, the most common malignancy, with 80% of cases being non–small cell lung cancer (NSCLC). More than three quarters of all cases of NSCLC present with locally advanced or widespread metastatic disease (stages IIIB and IV). Chemotherapy is the mainstay of treatment; however, overall survival following treatment is poor and has not significantly improved in over two decades. No single chemotherapy combination has shown significantly superior efficacy to any other, suggesting a therapeutic ceiling. The mechanism of death and pharmacodynamics of chemotherapy, relies on efficient initiation of programmed cell death or apoptosis (2–4). This process is an elaborate, biochemically stereotyped, phylogenetically conserved, and energy-requiring process that once engaged, irreversibly commits the cell to undergo self-destruction. In general terms, anticancer efficacy of chemotherapy correlates with intrinsic apoptosis sensitivity, most evident in the chemocurable germ cell cancers and lymphomas.

Apoptosis resistance is a fundamental property of many solid tumors and may play a critical role in determining tumor response phenotype following treatment with cytotoxic therapy. The low level of spontaneous apoptosis (5, 6) and general resistance of NSCLC cells to a diversity of cytotoxic modalities including radiotherapy suggest a defect in intrinsic apoptosis signaling (7).

The recent growth in understanding of the core apoptosis machinery of the cell has provided a framework for understanding how apoptosis is regulated in NSCLC cells. A critical event during apoptosis involves proteolytic activation of a constitutively expressed family of cytoplasmic zymogens with aspartate-specific cysteine protease activity, termed caspases. These enzymes play a critical role in executing the final common pathway of apoptosis. It may be hypothesized that caspases are targets for suppression in NSCLC, and that this may in part, account for the apoptosis resistant phenotype.

Two distinct but converging pathways have been described, which lead to caspase activation. The mitochondrial or intrinsic pathway transduces cytotoxic signals arising from within the cell (e.g., following DNA damage). Second, cell surface death receptors trigger the extrinsic pathway, which directly mediates caspase activation following ligation by endogenous ligands that include FAS ligand, tumor necrosis factor α, tumor necrosis factor β, tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), and Apo 3 ligand. There is crosstalk between both extrinsic and intrinsic pathways such that mitochondrial signaling can amplify signals arising from death receptor ligation in experimental models. Caspase inhibitors block the effects of a diverse range of cytotoxic drugs, reflecting the essential role of caspases in mediating their anticancer activity.

Teleologically, inhibition of caspases in cancer cells is a potentially efficient mechanism to both promote cell survival during tumorigenesis and contribute to chemoresistance. Elucidating the regulation of caspases in NSCLC is therefore essential to understand the molecular basis of the apoptosis resistant phenotype, and second, to identify novel molecular targets and strategies for improving upon current therapy (8).

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Mitochondria play a critical role in integrating proapoptotic death signals. This is achieved via mitochondria-to-cytosol signaling, involving the release of apoptogenic factors during mitochondrial outer membrane permeabilization (MOMP). In response to cell damage (e.g., multidomain proapoptotic proteins of the BCL-2 family), BAX and BAK are activated during the premitochondrial phase of apoptosis. BAX oligomerizes and translocates to the mitochondrial outer membrane, whereas BAK is activated within the outer mitochondrial membrane, leading to MOMP, mitochondrial inner membrane permeabilization, and apoptogenic factor release (9–11). Following DNA damage, MOMP is induced by BAX-dependent activation via the BH3 proapoptotic protein PUMA, induced by p53 and p73 (12, 13). BAX-independent MOMP is mediated by translocation of histone H1.2 (14).

Apoptogenic factors act in parallel to mediate caspase-dependent and caspase-independent cell death and include cytochrome c (15), apoptosis-inducing factor (16, 17), OMI/HtrA2 (18), smac/DIABLO, endonuclease G, and caspase-activated DNase (19). During MOMP, caspase 2 (CASP2) and CASP9 are released, accompanied by many other proteins (20). The release of apoptogenic factors is a key, rate-limiting step in apoptosis induction.

The assembly of a heterotrimeric complex comprising cytochrome c, APAF-1, and CASP9, termed the apoptosome (21–23), is shown in Fig. 1. This results in the autoproteolytic processing and activation of the apical or initiator CASP9, resulting in downstream activation of key executioners CASP3 and CASP7. CASP3/7 can amplify CASP9 activity via a feedback loop mechanism, cleaving CASP9. The protein Bcl-XE5 has been shown to interact directly with APAF-1 leading to inhibition of procaspase 9 activation (24) and is expressed in several cancers but not normal lung tissue.

NSCLC cell lines express APAF-1 and procaspase 3 at high level and with normal levels of CASP9 and CASP7 compared with normal lung tissue (25). It has been suggested that APAF-1 and procaspase 3 are functional and overexpressed in NSCLC, and that cytosolic inhibition of CASP3 activation, inhibits apoptosis-driven apoptosis. However, evidence suggests that apical caspase dysregulation may suppress apoptosis function. Primary NSCLC and cell lines express the apoptosis inhibitor and splice-variant, CASP9b, with a high CASP9b/CASP9 ratio. Gemcitabine (2',2'difluorodeoxycytidine, a deoxynucleoside analogue), a commonly chemotherapeutic drug in the first-line treatment of NSCLC, regulates CASP9 RNA splicing and decreases the level of CASP9b with concomitant increase in endogenous ceramide generated via the sphingolipid pathway. Inhibitors of ceramide block this process. Procaspase 9 also undergoes post-translational modification and is inhibited by phosphorylation on Ser196, mediated by AKT/protein kinase B (26). However, the prevalence and significance of procaspase 9 (Ser196) phosphorylation status in NSCLC has not yet been explored.

In vivo Expression and Prognostic Significance of Caspase 3 in Non–Small Cell Lung Cancer. CASP3 is directly activated following cytosolic assembly of the apoptosome. CASP3 and CASP7 are substrates for CASP9 and are proteolytically activated by terminal cleavage and assembly of a tetrameric enzyme resulting in formation of an active site (Fig. 2). CASP3 plays an important role in cytotoxic drug-induced apoptosis in NSCLC (e.g., by paclitaxel; ref. 27). Upon activation, CASP3 localizes to the nucleus during apoptosis; this is observed in SCLC cells but not in NSCLC cells (28).

In vitro, chemotherapy-resistant NSCLC is associated with reduced expression of apical CASP9 and CASP3 as measured by immunohistochemistry (29); this suggests constitutive block of signaling via the apoptosome in NSCLC. Ineffective activation of the final common pathway of apoptosis may contribute to drug resistance in NSCLC.

![Fig. 1](https://example.com/fig1.png) The release of cytochrome c during MOMP activates cytosolic assembly of the APAF-1 apoptosome in a dATP-dependent manner. Oligomerized APAF-1/cytochrome forms a scaffold for binding of proc-CASP9, resulting in its autocataytic cleavage and activation. Activation of downstream caspases. IAPs prevent caspase activation downstream of the apoptosome. MOMP is accompanied by release of IAP inhibitors SMAC/diablo and OMI/HtrA2; apoptosis-inducing factor is also released.
significantly to the observed drug and radiotherapy resistance observed in the clinic. Reestablishment of expression of CASP8 and CASP9 by transfection of human xenografts in nude mice, strongly induces apoptosis and mediates sensitization to cisplatin-induced cell death.

The in vivo expression level of CASP3 in NSCLC has been shown to correlate with survival. In a series of 151 studies, Koomagi and Volm measured the absolute level of CASP3 and found a correlation between CASP3 level and lymph node metastases. Patients with low levels of CASP3 had worse overall prognosis (30). Inverse correlation between CASP3 expression and angiogenesis measured by microvessel density in vivo suggests prosurvival effects of hypoxia in NSCLC (30). Reduced expression and defective function of CASP3 could arise from mutations in the coding region. However, analysis of somatic mutation of CASP3 by Soung et al. has shown that this occurs relatively infrequently in NSCLC. In a study involving sequencing of all coding regions and splice sites of the CASP3 gene, 4 of 181 tumor specimens exhibited somatic mutations (2.2%; ref. 31); a low frequency of mutations was also seen in other cancers including breast, colon, stomach, bladder, and leukemia. In summary therefore, CASP3 down-regulation may be a relevant mechanism contributing to apoptosis resistance, although this is not commonly associated with somatic mutation.

**Inhibitor of Apoptosis Proteins in Non–Small Cell Lung Cancer.** The inhibitor of apoptosis (IAP) family of proteins were originally identified in baculoviruses and facilitate viral propagation by antagonizing host cell apoptosis defense mechanisms. Mammalian homologues of viral IAPs expressed in NSCLC include X-linked inhibitor of apoptosis protein (XIAP and BIRC4), survivin (BIRC5), cIAP1 (hIAP2 and BIRC2), and cIAP2 (hIAP1 and BIRC3). IAPs exhibit structural similarity, sharing one or more 70 amino acid long baculoviral IAP repeat domains and are members of a larger baculoviral IAP repeat protein family. Determination of the structural basis of IAP/caspase interactions and their inhibition by endogenous IAP inhibitors (smac/DIABLO and OMI/HtrA2) presents an opportunity for novel drug development and exploitation in NSCLC (32, 33).

**Expression and Prognostic Significance of XIAP in Non–Small Cell Lung Cancer.** XIAP is an antiapoptotic baculoviral IAP repeat protein that efficiently inhibits activated caspase 3, caspase 7, and caspase 9 (34). The BIR3 domain of XIAP interacts with activated (processed) CASP9 via its terminal p10 subunit which becomes exposed upon activation and is one of the most potent IAPs. CASP3 and CASP7 are inhibited by the linker region after BIR1 and BIR2 domains of XIAP, such that CASP3 is inactivated in a complex with the apoptosome (35). Survivin stabilizes XIAP via formation of an IAP-IAP complex that protects XIAP from ubiquitination and proteosome degradation (36).

Yang et al. have shown apoptosis-deficient induction of caspase activation in NSCLC cells in vitro, without evidence of loss of apoptosome components, via a process involving constitutive binding of XIAP to processed CASP9 (37). XIAP specific down-regulation by antisense oligonucleotide (G4) mediating 60% reduction in XIAP mRNA has been shown to sensitize NSCLC cells in vitro to doxorubicin, taxol, vinorelbine, and etoposide. In vivo, XIAP-specific antisense oligonucleotides delayed tumor engraftment in H460 xenografts to vinorelbine, taxol, doxorubicin, and etoposide (38) to a greater degree than either agent alone. These findings suggest that down-regulation of XIAP may be effective in significantly altering apoptosis phenotype. Hu et al. have shown that inhibition doxorubicin and taxol mediate apoptosis in NSCLC cells via a process associated with down-regulation of XIAP and phosphorylation of BCL-2, without changing levels of BCL-XL expression (38). This effect on XIAP is concentration dependent and mimicked by the mitogen-activated protein kinase kinase–specific inhibitor U0126 and the PKCα inhibitor, staurosporine.
The constitutive level of XIAP expression has been shown not to predict response to chemotherapy in vitro (39). Similar levels of XIAP have been observed in etoposide or irradiation sensitive and insensitive NSCLC cell lines, respectively, suggesting that the constitutive level of XIAP alone is not a predictor of apoptosis threshold in a manner that influences sensitivity to cytotoxic therapy, at least in vitro (40, 41).

In vivo, Giaccone’s group has shown that XIAP is expressed in NSCLC (42) and is expressed at a lower level than in small cell lung cancer (40). In a series of 144 patients with early stage NSCLC, 61 were considered to have high expression of XIAP. No correlation with apoptotic index was observed; however, there was inverse correlation with the proliferation antigen ki67 and mitotic index. High XIAP expressing NSCLC tumors were shown to be associated with longer survival of patients when analyzed by both univariate Kaplan-Meier analysis and multivariate Cox regression. The authors suggested that this paradoxical inverse prognostic relationship for XIAP implicates a more complex biology of XIAP in vivo compared with observations in vitro. Interestingly, XIAP has been shown to be an adverse prognostic factor in renal cell cancer (43), and the ratio of XIAP to its endogenous inhibitor SMAC/DIABLO increases during its progression, implicating significant differences in biology compared with NSCLC (44). Evidence also suggests that XIAP may be a useful therapeutic target in prostate cancer (45).

Expression and Prognostic Significance of Survivin in Non-Small Cell Lung Cancer. Survivin is an IAP that is significantly overexpressed in cancers compared with normal adult tissues (46) and inhibits apoptosis following ligation of death receptor, activation of BAX or caspases, and cytotoxic drugs (47). Survivin is expressed in a cell cycle-specific manner in the G2-M phase and associates with the microtubules of the mitotic spindle at the beginning of mitosis. This microtubule interaction is saturable, and if disrupted is a predictor of apoptosis threshold in a manner that influences sensitivity to chemotherapy (48). Survivin mRNA has been used to detect bladder cancer and is a poor prognostic factor for several cancers including the breast (49, 50) and pancreas (51).

Survivin mRNA expression in patients with NSCLC (stages I-IIIA), undergoing radical surgery, has been shown to be positively correlated with worse overall survival. In one series, expression was prevalent in 85% of tumors (52). In a Chinese series of 76 patients, survivin mRNA expression also predicted worse survival (53); expression in NSCLC tissue was higher (61%) compared with normal tissue (19%). No association with histology, stage, nor lymph node metastases was identified; however, molecular correlation was seen with c-myc and p53 but not K-ras (53).

Falleni et al. have shown that survivin is preferentially expressed in early-stage NSCLC (54). Survivin mRNA transcript and protein levels were studied in a series of 83 NSCLC samples from patients with stage IA and IB disease. Interestingly, survivin was seen in both nonmalignant and malignant tissue; however, expression was greater in the latter. Survivin has also been identified as a predictive factor, associated with reduced local control of stage IIIA NSCLC by radiotherapy (55).

In vitro, antisense oligonucleotide mediated down-regulation of survivin and is associated with increased caspase activity and sequence-specific sensitization of NSCLC cells to etoposide (56). Altieri’s group have explored the apoptosis-modifying activity of a replication deficient adenovirus construct (pAd-T34A) encoding a mutant, nonphosphorylatable survivin (Thr24 → Ala) both in vitro and in vivo (57). They showed that pAd-T34A induces apoptosis selectively in lung cancer and malignant cell lines but not in normal cells by a mechanism involving increased apoptosome-dependent apoptosis. Chemosensitization in vitro was also observed suggesting therapeutic potential for enhancement of tumor cytotoxicity by disruption of survivin signaling.

Survivin expression in NSCLC cells correlates in vitro with expression of cyclooxygenase 2 (COX2), an enzyme associated with increased invasiveness, angiogenesis, and apoptosis resistance (58). COX2 expression is an independent prognostic factor for poor prognosis in patients with NSCLC (59) and directly stabilizes the expression of survivin by modulating its ubiquitination. This effect can be achieved by exogenous treatment of NSCLC cells with prostaglandin E2 (58). Krysan et al. have shown that human xenografts in nude mice exhibit decreased survivin levels following transfection with antisense COX2, whereas sense COX2 transfection is associated with increased survivin expression (58). These findings suggest a direct relationship between IAP expression and COX2. Therapeutic inhibitors of COX2 are being evaluated in clinical trials (60); however, their ability to modulate in vivo NSCLC survivin expression has yet to be established.

A recent study by Zhang et al. has shown that survivin is down-regulated by a therapeutic dose of nonradioactive iodide that induces apoptosis in lung cancer cells transfected with the sodium iodide symporter and thyroperoxidase. This effect is associated with overexpression of CDKN1A (p21/waf-1). In vivo antitumor activity of nonradioactive iodide was observed following i.p. administration of sodium iodide in severely combined immunodeficient mice bearing sodium iodide symporter/thyroperoxidase–transfected human lung cancer xenografts (61).

Survivin down-regulation and apoptosis is induced in NSCLC cells in vitro by the ribosomal protein S29 (62); the mechanism also involves reduction of BCL-2 family proteins BCL-2 and BCL-XL (62). Proapoptotic BCL-2 proteins BAX and p53 are up-regulated concurrently with activation of apical caspases, CASP8/CASP9 and CASP3 and release of cytochrome c in a manner that does not involve mitochondrial inner membrane permeabilization, but involves release of apoptosis-inducing factor (62).

clAP-1 and clAP-2 in Non-Small Cell Lung Cancer. The IAPs clAP1 and clAP2 block CASP3 and CASP7 (63). These proteins are the only IAPs with caspase activation recruitment domains, also present in adaptor proteins such as APAF-1 and FADD. The caspase activation recruitment domain–dependent function of clAP1 and clAP2 is not well defined. Hofman et al. have examined the expression of clAP1 and clAP2 in a series of 34 tumor NSCLC specimens (64). Increase in clAP2 mRNA was observed, particularly in adenocarcinoma, whereas clAP1 mRNA was expressed at identical level in tumor specimens and control. Expression of clAP2 was observed mainly in low tumor-node-metastasis
adenocarcinomas (stage I with a 289% increase in expression), versus stages III/IV (with a 40% increase in expression). These findings therefore suggest that cIAP2 expression is increased in NSCLC but is preferentially expressed at high level in low stage adenocarcinoma.

Expression of cIAP1 is directly regulated by chemotherapy. Gemcitabine induces apoptosis and S-phase cell cycle arrest and radiosensitizes NSCLC via a caspase-dependent mechanism (65). Bandala et al. have shown that gemcitabine induces expression of cIAP1 via increase in the activity of the antiapoptotic transcription factor nuclear factor-κB (NF-κB) with concomitant down-regulation of Ik-B-α (66). NF-κB activity was shown to correlate with cIAP-1 expression. Disruption of NF-κB by overexpression of dominant-negative mutant of Ik-B-α sensitizes NSCLC cells to gemcitabine, an effect that is abrogated by overexpression of cIAP-1 (66), suggesting an important adaptive survival role for cIAP-1 during treatment of NSCLC cells with gemcitabine. A similar effect of chemotherapy on expression of NF-κB has been observed with Adriamycin in lung cancer cell lines (67).

Antagonism of Inhibitor of Apoptosis and its Exploitation in Non–Small Cell Lung Cancer. Suppression of IAPs may be an important strategy for cancer therapy (68–70). Inhibition of XIAP and survivin has been shown to sensitize pancreatic, breast, and colon cancer cell lines to apoptotic stimuli (71). The structural basis of the interaction between IAPs and caspases has been explored, and inhibition of IAP/caspase interactions presents an attractive strategy for therapeutic derepression of caspases. Overexpressed IAPs in NSCLC and their relation to dysregulated expression of CASP3 and CASP9 in NSCLC are summarized in Fig. 3.

Second mitochondria-derived activator (smac/diablo; ref. 72) and OMI/HtrA2 (18) are an apoptogenic proteins coreleased from mitochondria along with cytochrome c during MOMP. Smac/diablo directly competes with the binding site of XIAP localized to the p10 subunit of activated CASP9 and therefore disrupts the inhibitory interaction between CASP9 and IAP (35). Smac mRNA transcript is expressed at higher levels in primary squamous cell lung cancer compared with adenocarcinoma and in smokers compared with nonsmokers (73). Lack of smac expression is also associated with worse prognosis in NSCLC as measured by real-time PCR (73), and suggests an association among proapoptotic smac/diablo-mediated IAP derepression, caspase activation, and survival.

Pharmacologic inhibition of IAPs may be achieved by exploiting smac/diablo’s interaction with IAPs in NSCLC in vitro. Polyarginine conjugated smac peptide (smacN7) has been shown by Yang et al. to increase NSCLC cell apoptosis sensitivity, but not normal lung fibroblasts expressing modest amounts of IAP. In vivo, a modified smac peptide [smacN7(R)8] inhibited NSCLC lung cancer xenograft growth in combination with chemotherapy in vivo (37).

Glover et al. have reported a high throughput screening assay for “terapeptidomimetics” of smac/diablo involving detection of changes in fluorescence polarization produced by the binding of smac NH2-terminal tetra-peptide to the BIR3 domain of XIAP (74). By screening thousands of compounds, it has been possible to identify new lead pharmacophores with specific binding affinity to XIAP-BIR3.

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Upon ligation, death receptors interact with a wide variety of adapter proteins via a homologous death domain, that include FADD and CRADD. CASP8 and CASP10 are recruited to the adaptor protein via homologous interaction of a death effector domain (75) required for death signal

Fig. 3  Alterations of expression of IAP proteins and caspases in NSCLC. Up-regulation of the IAPs survivin, XIAP, and cIAP1/2 may confer resistance to apoptosome activation. Survivin binds and stabilizes XIAP. COX2 is upregulated in NSCLC and stabilizes survivin. Suppression of apoptosis may be mediated by expression of the apical isoform caspase 9b (which is suppressed at the level of RNA processing by gemcitabine). CASP9 and CASP7 are not overexpressed. However, the significance of Ser196 phosphorylation and inhibition of CASP9 is unknown. Extrinsic pathway signaling involving CASP8 seems important for apoptosis of NSCLC cells by gemcitabine, topotecan, or cisplatin. The significance of cFLIP expression in NSCLC in vivo is unclear.
transduction. The complex of death receptor, adaptor protein, and apical caspase is termed the death-inducing signaling complex or DISC, and its assembly is necessary for apical CASP8 signaling following death receptor ligation. Crosstalk with the mitochondrial pathway occurs via processing and truncation of the BH3 only BCL-2 family proapoptotic BID results in BAX-dependent MOMP. This pathway therefore amplifies apoptosis via the apoptosome. Death receptor activation of the caspase pathway presents a novel strategy for facilitating chemotherapy-induced apoptosis and has been explored in NSCLC. Thus, TRAIL receptors DR4 and DR5 are currently being explored as targets for induction of apoptosis in solid tumors.

**Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand Receptors Are Expressed in Primary Non–Small Cell Lung Cancer.** Primary NSCLC cells express significant levels of TRAIL. De Jong’s group has explored the expression of TRAIL receptors in primary NSCLC specimens extracted at bronchoscopy from patients with stage III disease (76). DR4 was expressed in 99% of cases, DR5 in 82%, and TRAIL in 91%. Immunostaining of DR4 and DR5 was predominant in the basal cells and reduced or absent in the mature cells; the converse was observed for TRAIL. Strong DR4/DR5 expression was seen in areas of poor differentiation. The expression of DR5 was found to be an adverse prognostic factor associated with a significantly increased risk of death (odds ratio, 5.74).

The TRAIL receptor is localized to 8p21-22, a region that is commonly deleted in lung cancer (77). The entire coding region of DR5 has been explored by Lee et al. in 107 NSCLC specimens (77). Mutations in the death domain were determined in 10% of cases, with potential to disrupt DISC signaling in a minority of patients. Mutations in DR5 are relatively uncommon in vivo; one study by Wu et al. failed to show mutations in DR5 coding region in 100 primary NSCLC tumors (78).

Mutational screening of DR4 in 17 NSCLC cell lines and 24 primary NSCLC specimens has revealed two misense mutations in the receptor’s ectodomain. One nucleotide substitution results in exchange of cytosine 626 for guanine (C626G) converting an arginine to threonine; the other converts guanine 422 for adenine (G422A), changing a histidine to an arginine (79). The frequency of homozygous alleles was 35% in primary lung cancer samples, and the mutations were localized to the ligand-binding domain based on the crystal structure of DR5 and its homology of DR4.

TRAIL receptors represent drugable targets with therapeutic relevance. Clinical trials are currently in progress to evaluate the efficacy of agonistic monoclonal antibodies targeting TRAIL in patients with cancer. In experimental model systems, TRAIL-induced apoptosis is highly selective for malignant versus normal cells, suggesting potential for low therapeutic index (80). Hepatotoxicity, may represent a dose-limiting toxicity as indicated by studies in nude mice (81–83).

**Chemotherapy Evokes Death-Inducing Signaling Complex–Independent CASP8 Activation in Non–Small Cell Lung Cancer.** Giaccone’s group has explored the function of apical caspases within the extrinsic pathway using stably expressed caspase inhibitors in NSCLC cell lines (84). Gemcitabine, cisplatin, and topotecan induced apoptosis in the NCI-N460 cell line in a manner that was not inhibited by expression of the intrinsic pathway inhibitors XIAP and CASP9S. In contrast, expression of dominant-negative CASP8 or cytokine response modifier A significantly reduced chemotherapy-induced apoptosis and promoted clonogenic survival. Expression of dominant-negative FADD did not inhibit chemotherapy-induced activation of CASP8, implicating a direct activation independent of the DISC. Despite the involvement of CASP8 in cytotoxic drug-induced apoptosis, FAS and the CD95 pathway do not seem to play a role (85).

CASP8, FAS, FAS ligand, and DR5 have been shown to be frequently down-regulated in small cell lung cancer but not NSCLC (86). DNA methylation of CpG islands is associated with CASP8 gene silencing in SCLC but not NSCLC (87). CASP10 is down-regulated at the protein level in NSCLC, with potential for reduced sensitivity to death receptor ligands. FLIPs are endogenous antagonists of DISC associated caspase activation and inhibits signal transduction mediated by death receptors; however, the expression of FLIP has not been characterized in NSCLC in vivo.

**Exploiting Extrinsic Pathway Signaling in Non–Small Cell Lung Cancer.** The lack of DNA methylation of CASP8 in NSCLC compared with SCLC, coupled to the probable requirement for CASP8 activation during chemotherapy-induced apoptosis suggests that strategies employing activation of CASP8 via the DISC for example, could be used to overcome apoptosis resistance in NSCLC. Some NSCLC cell lines exhibit sensitivity to TRAIL induced apoptosis, which is inhibited by the apoptosis inhibitor BCL-2 (88).

In combination with chemotherapy, TRAIL is synergistic (89), and this effect does not correlate with either CASP8 nor FLIP expression level (90). Simultaneous expression of TRAIL and p53 increases apoptosis sensitivity in NSCLC cell lines (91). Up-regulation of TRAIL receptors by the synthetic retinoid 6-(3-(1-adamantyl)-4-hydroxyphenyl)-2-naphthalene carboxylic acid (CD437) sensitizes NSCLC to apoptosis associated with wild-type p53 but not normal epithelium (92). As such, TRAIL receptor density regulates signal transduction via the DISC, and the efficiency of extrinsic pathway-dependent caspase activation.

The diterpine triepoxide, PG490 (triptolide) extracted from the Chinese herb *Tripterygium wilfordii* induces apoptosis in NSCLC cells but not normal bronchial epithelium in a manner that requires activation of CASP8 and CASP3 but not CASP9 (93). PG490 sensitizes cancer cells lines and primary NSCLC cells to TRAIL (93). The synergy between TRAIL and PG490 involves activation of the mitogen-activated protein kinase pathway and is inhibited by the extracellular signal-regulated kinase inhibitor U0126 but not by the p38 pathway inhibitor SB203580, implicating ERK2 in mediating the interaction. Triptolide in combination with TRAIL also inhibits NF-κB activity, and this may contribute to apoptosis induction (94); the combination blocks trans-activation of p65 and is potentiated by MG132 which blocks NF-κB activation by inhibiting degradation of IκBα. Triptolide is currently in clinical development and may have therapeutic potential for treatment of NSCLC by enhancing CASP8 function.
CONCLUDING REMARKS

It is widely accepted that conventional cytotoxic therapy for NSCLC has reached a therapeutic plateau. Historically, cytotoxic drug efficacy has correlated with tumor cell kill across the spectrum of both hematological and solid tumors and implicates a dependence on intrinsic apoptosis sensitivity. NSCLC cells are apoptosis resistant, a process that may arise due to defective signaling in the final common apoptosis pathway requiring caspases. Taken together, current evidence strongly suggests that caspase activation may be constitutively defective. Consequently, strategies to derepress endogenous caspase inhibitors in NSCLC suggest promising activity in model systems and present a rational and entirely novel strategy for improving treatment in this commonly fatal malignancy.

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