MINIREVIEW

Apoptosis, Chemoresistance, and Breast Cancer: Insights From the MCF-7 Cell Model System

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The MCF-7 cell line was derived from a patient with metastatic breast cancer in 1970. Since then it has become a prominent model system for the study of estrogen receptor–positive breast cancer. With this model as a focus, this review summarizes important studies addressing tumor necrosis factor-α as a prototypical apoptosis-inducing cytokine in MCF-7 cells. Both survival and death receptor signaling pathways are discussed in terms of their role in chemotherapy-induced apoptosis as well as in chemoresistance. Novel therapeutic approaches to the treatment of breast cancer are proposed utilizing knowledge of these signaling pathways as targets. Specifically, ceramide metabolism is proposed as a novel target for chemosensitivity, perhaps combined with selective inhibitors of Bcl-2 or PI3K/Akt/nuclear factor-κB. Suggested areas of future research include translational studies manipulating candidate survival and death signaling pathways. Exp Biol Med 228:995–1003, 2003

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Breast Cancer and the Role of Apoptosis

Breast cancer is the most common malignancy in American and northwestern European women (1–3). It is estimated that one in eight American women and one in 12 women in the United Kingdom will develop breast cancer in their lifetime and the incidence rates in industrialized nations are on the rise (4). Approximately one-third of women with breast cancer develop metastases and ultimately die from the disease. The most common sites for the development of metastases, excluding lymph nodes, are bone, liver, and lung (1). Men are also susceptible to the disease with an estimated 1500 cases and 270 deaths per year in the United States (4).

Breast epithelial cell homeostasis requires the balance of cell proliferation with a type of cell death called apoptosis (5). Apoptosis is defined as genetically programmed autonomous cell death, and it occurs in healthy breast cells at varying rates during the estrus cycle in response to changes in hormone levels (5). Apoptosis is also regulated by nonhormonal signals. Changes in the genetics of apoptotic regulatory mechanisms may result in an increase in cell numbers, as well as the preservation of genetically altered cells, which begins the process of tumorigenesis (6). Apoptosis contributes to cell death in tumors treated with various anticancer agents. Chemotherapy, radiation therapy, and immunotherapy all rely heavily on apoptosis to kill breast cancer cells (7, 8). Despite the fact that many tumors initially respond to therapy, cells can subsequently survive and gain resistance to these treatments. This leads to a more phenotypically aggressive cell variant with an inclination to metastasize. Chemoresistance often accompanies the progression of breast cancers from a hormone-dependent, nonmetastatic, antiestrogen-sensitive phenotype to a hormone-independent, invasive, metastatic, antiestrogen-resistant phenotype (9). The answer to iatrogenically induced hormone and chemotherapy-resistant cancer cells remains elusive, because a solid understanding of breast cancer biology

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is still incomplete. The estrogen receptor (ER)-positive MCF-7 cell line has been studied longer than any other breast cancer cell model system and is the focus of this review. In addition to providing the basic tool for distinguishing ER-positive from ER-negative breast cancer tumors, specific key pro- and antiapoptotic regulators in the signal transduction pathways that influence chemoresistance have recently been identified in this system. Continuing biological insights learned from this *in vitro* cell model are predicted to provide novel approaches to cancer therapy within a few years.

The MCF-7 Cell Line as a Model System for the Study of Apoptosis. In 1970, the MCF-7 cell line was derived from a pleural effusion of a patient with metastatic breast cancer (10). Levenson and Jordan (10) and Soule *et al.* (11) led the research that established MCF-7 cells as the first hormone-responsive breast cancer cell line. Extensive research on MCF-7 cells in the 1970s and 1980s led to the development of monoclonal antibodies to the ER, which facilitated the cloning and sequencing of the ER, the development of techniques to quantify the estrogen and progesterone receptors in breast tumor, and a significant reduction in the occurrence of false negative results for ER status in tumors in patients receiving long-term tamoxifen therapy; tamoxifen occupies the ER causing false negative results using ligand-binding assays (10). The usefulness of the MCF-7 cell line as an investigative tool led to its adoption in laboratories worldwide. Several decades of use in independent laboratories have facilitated the evolution of distinct MCF-7 lineages (12, 13). Documented differences include the ability to undergo DNA fragmentation, differential sensitivities to estrogens and antiestrogens, differential expression of ER, ER mRNA, and progesterone receptor, and differences in tumorigenicity and proliferation rates. In 1998, our laboratory reported that discrepancies exist concerning the apoptotic responses of MCF-7 cells to the apoptosis- inducing agents tumor necrosis factor-α (TNF-α) and anti-Fas antibody. Subsequently, differences in apoptotic resistance were extended to doxorubicin. We reasoned that the MCF-7 variants are, in many ways, analogous to the *in vivo* apoptotic-resistant aggressive phenotypes induced by cancer therapy (13). What follows is a summary of the current relevant data for our contention that MCF-7 cells continue to serve as an excellent *in vitro* model for studying the mechanisms of chemoresistance as it relates to susceptibility to apoptosis.

Apoptosis: Mechanisms and Regulation

Cellular and Biochemical Features of Apoptosis. Apoptosis, from the Greek word for "falling off" or "dropping off" as leaves from a tree, is an essential part of the development and maintenance of life (5). It was known for some time that portions of the developing embryo underwent cell death, but apoptosis was not characterized until 1972 when Kerr *et al.* (14) published their research recognizing apoptosis as an occurrence in adults relevant to health and disease. Apoptosis is now defined as a discrete sequence of morphological changes resulting in cell death with extensive dsDNA cleavage accompanied by chromatin compaction and segregation along the nuclear membrane (6). Mild convolution of the cell membrane and shrinkage of the cell lead to membrane blebbing and fragmentation. During this process, the membranes are well maintained and do not release their contents or influence behavior of adjacent cells. Resulting cell fragments, or apoptotic bodies, are phagocytosed by surrounding cells (5). The biochemical complement to these morphological changes is a complex and murky amalgam of signal transduction cascades that leads to the activation of caspases, which are proteases produced as zymogens, that cleave various cell proteins causing the death of the cell (15). The molecular events of apoptosis can be divided into three steps: 1) initiation by an apoptosis-inducing agent, 2) activation of the caspases by a signal transduction cascade, and 3) proteolytic cleavage of cellular components. Many death and survival genes, which are regulated by extracellular factors, are involved in apoptosis. Early events of apoptotic signaling at the membrane level involve ceramide generation through activation of sphingomyelinase (SMase) (16) and downstream signaling involving BCL-2 family members (17), the inhibitor of apoptosis (IAP) family of proteins (18, 19), the transcription factor nuclear factor-κB (NF-κB) (20), members of the mitogen-activated protein kinase (MAPK) family (21), such as p42/44MAPKs (extracellular signal-related kinases [ERKs]), SAPK/JNK, and p38MAPK, and caspases (22). There appears to be a critical set of early apoptotic signals, regardless of initiating agent, and their subsequent regulation by survival signals. The apoptotic threshold of a cell is determined by the balance between pro- and antiapoptotic regulators expressed in the cell, as illustrated in Figure 1 (5). The proapoptotic and prosurvival pathways are interconnected such that manipulation of one can cause changes in the other.

Role of Death Receptor Signaling in Chemotherapy-Induced Apoptosis. Because numerous studies to date strongly suggest the induction of apoptosis as the means by which chemotherapeutic drugs kill target breast cancer cells, there are several important implications for the future of breast cancer chemotherapy. The efficacy of a variety of unrelated chemotherapeutic drugs can be modified based on biochemical alterations that make cells more or less susceptible to apoptosis. Changes that decrease the ability of cancer cells to activate the apoptotic machinery may play a role in chemoresistance to a wide variety of drugs. A detailed understanding of the apoptotic signal transduction machinery will be necessary to thoroughly evaluate the importance of this concept in the design of more effective chemotherapy (23). A number of studies have suggested that anticancer drugs kill susceptible breast cancer cells by inducing the expression of death receptor ligands, such as Fas ligand (FasL) (24, 25). In an autocrine or paracrine fashion, FasL then binds to Fas and activates

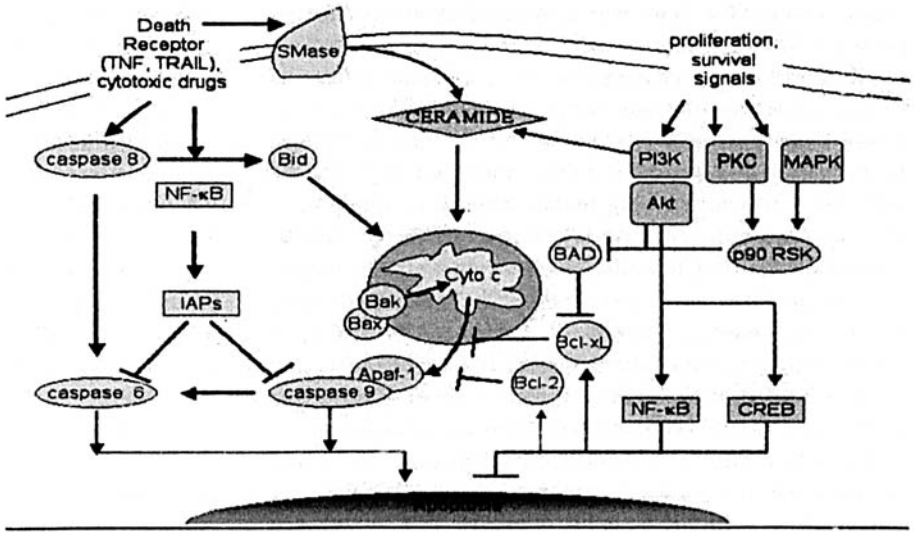

Figure 1. Pro- and antiapoptotic pathways. The death receptor ligands, such as TNF and TRAIL, initiate proapoptotic pathways, such as sphingomyelinase activation, leading to ceramide and cytochrome c release from mitochondria. In addition, caspase-8 and NF-κB can be concurrently activated, leading to simultaneous stimulation of both apoptotic and antiapoptotic signals. The relative constitutive expression of antiapoptotic pathways such as PI3K, PKC, and the MAPKs influences the efficacy of proapoptotic signals.

the death receptor pathway via caspase 8 (26). FasL is expressed on breast tumors, as are TNF-α receptors and receptors for TNF-related apoptosis-inducing ligand (TRAIL) (27). The role of FasL/Fas (CD95) has been controversial due to discrepancies in cell lines used, kinetics, cell culture conditions, drug concentrations (suprapharmacological concentrations of drugs may activate other, Fas-independent pathways), and concentration and effectiveness of blocking reagents (28). However, evidence to date leads to the conclusion that the mitochondrial pathway plays a more predominant role in drug-induced apoptosis, with the exception of 5-fluorouracil. This drug whose active metabolite is 5-fluoro-2′-deoxyuridine-5′-monophosphate inhibits thymidylate synthase and causes thymine depletion. Thymine depletion or fluorouracil treatment results in a p53-dependent increase in expression of FasL in human colon cancer cell lines (29). Additional studies are needed in breast cancer cell lines to determine the precise role of the Fas/FasL pathway. This pathway was discovered before the cytochrome c/Apaf-1/caspase-9 pathway, and for the majority of anticancer drugs this latter pathway is most often invoked.

Tumor Necrosis Factor-α as a Prototypical Apoptosis-Inducing Cytokine. TNF-α is a naturally occurring cytokine secreted by cells of the immune and other systems. Although TNF-α is cytotoxic to some tumor cells, it is rarely cytotoxic to normal cells. This unique property has led to numerous studies of TNF as a chemotherapeutic agent and apoptosis-inducing agent. However, the efficacy of TNF as a chemotherapeutic agent is in question because systemic doses of TNF cause severe toxicities, namely hypotension, liver dysfunction, leukopenia, and thrombus formation (30). TNF family members, such as TRAIL, that minimize toxicity and augment chemotherapy-induced apoptosis are showing considerable promise, particularly in combination with classic chemotherapeutic drugs in breast cell lines (31). Binding of TNF and similar ligands, including TRAIL and Fas, to their respective receptors induces receptor trimerization and recruitment of adaptor proteins to the cytoplasmic death domain of the receptor. Upon binding, TNF can activate both apoptotic pathways and survival pathways inside the cell. TNF-α has two receptors, p55 (TNFR1) and p75 (TNFR2), with most apoptotic pathways mediated through p55 (15). Although p55 expression is necessary for TNF to produce apoptosis, it is not sufficient by itself (23, 28). Like most death ligands, postreceptor signaling events determine the sensitivity of a cell to TNF-induced apoptosis (23). Binding of TNF to p55 leads to recruitment of an intracellular death–inducing signaling complex (DISC) consisting of at least six different members: TRADD (TNFR1-associated death domain), TRAF1 and 2 (TNFR-associated protein 2), receptor interacting protein (RIP1 and 2, a death domain kinase), and RAIDD (receptor interacting protein [RIP]-associated ICH-1/CED-3-homologous protein with death domain) (32). DISC members have been shown to interact through the "death domains," which are conserved sequences on the amino terminus, intracellular portion of TNF and other death receptors (33–35). These death domains serve as docking sites for a group of intracellular protein mediators known as TNF-associated death domain proteins (TRADD), which have identical death domains in their carboxy terminals (33, 35). TRADD serves the purpose of binding to the TNFR1 and facilitates the binding of procaspase-8 via another unique, shared protein sequence termed the death effector domain (DED). Caspase-8 possesses a DED that binds to the DED portion of TRADD and, once bound, activates the procaspase (33). At this point the extrinsic and intrinsic caspase pathways merge as active caspase-8 catalyzes the conversion of effector procaspases and, ultimately, executioner caspases resulting in apoptosis (36). Lipid second messengers, numerous protein kinases, and transcription factors have been implicated in defective TNF death pathways, as well as other chemotherapeutic drugs (15, 37–39).

Caspases and Their Role in Apoptosis. The family of mammalian apoptotic proteases, now called

caspases, was first described when their nematode homologues were recognized in the worm *Caenorhabditis elegans*. Caspases are characterized by a cysteine active site with an aspartate substrate specificity. They exist as proenzymes comprised of a prodomain and a catalytic protease domain. Caspases are categorized as initiator (caspase-8,- 9, -10, and -12), which cleave other caspases, or executioner caspases (caspase-3, -6, and -7), which cleave various cellular proteins (36). Currently there are two known pathways that activate the caspase cascade (Fig. 2). The intrinsic pathway involves the Bcl-2 family of proteins and the release of cytochrome c from the mitochondria (40, 41). Mitochondria are implicated in apoptosis through several proposed mechanisms, and there is evidence that mitochondria serve as an early target in TNF-induced cytotoxicity (42). The steps leading to the release of cytochrome c from the mitochondria are poorly understood. However, it is known that in the intrinsic pathway cytochrome c activates the adaptor protein Apaf-1, which then activates procaspase-9 in a dATP-dependent reaction, which leads to the activation of downstream effector caspases (41). In addition, release of manganese superoxide dismutase (MnSOD) from the mitochondria, which catalyzes reactions reducing oxidative stress, is a general effect of TNF exposure. TNF-sensitive MCF-7 cells express low or undetectable levels of MnSOD, whereas resistant cells express relatively high levels (42). The extrinsic pathway is independent of mitochondria and is induced by death receptor–protein complexes, mentioned previously, that cleave procaspase-8. Cleavage and activation of caspase-8 result in initiation of the caspase cascade. Following initial induction, the intrinsic and extrinsic pathways merge at the level of the effector caspases. These effector caspases cleave DNA repair enzymes, intracellular signaling molecules, as well as cytoskeletal and nuclear proteins. Cross-talk between the two pathways is present but minimal. During such cross-talk, caspase-8 cleaves the proapoptotic cytosolic protein Bid, which translocates to the mitochondria and binds to Bad, another proapoptotic protein, resulting in the release of cytochrome c and activation of Apaf-1 (25). The MCF-7 cell line does not express caspase-3, a critical component of the caspase cascade, due to a deletion in the *casp-3* gene, suggesting the existence of caspase-3-independent apoptotic pathways (43). Liang *et al.* (44) showed that MCF-7 cells can undergo apoptosis by the sequential activation of caspases-9, -7, and -6. Although caspase-3 is not required for TNF-induced apoptosis, it is required for some of the typical morphological changes of apoptotic cells, such as DNA fragmentation, and DNA fragmentation is not always observed in TNF-induced apoptosis in MCF-7 cells (45, 46). The presence of caspase-3 can increase a cell's sensitivity to apoptosis-inducing agents. MCF-7 cells, which are relatively insensitive to many chemotherapeutic agents, acquire greater sensitivity to doxorubicin- and etoposide-induced apoptosis when caspase-3 is reconstituted (47). Future research may show whether maximizing cross-talk between the intrinsic and extrinsic pathways of apoptosis and upregulation of caspase-3 are potential targets for breast cancer therapy.

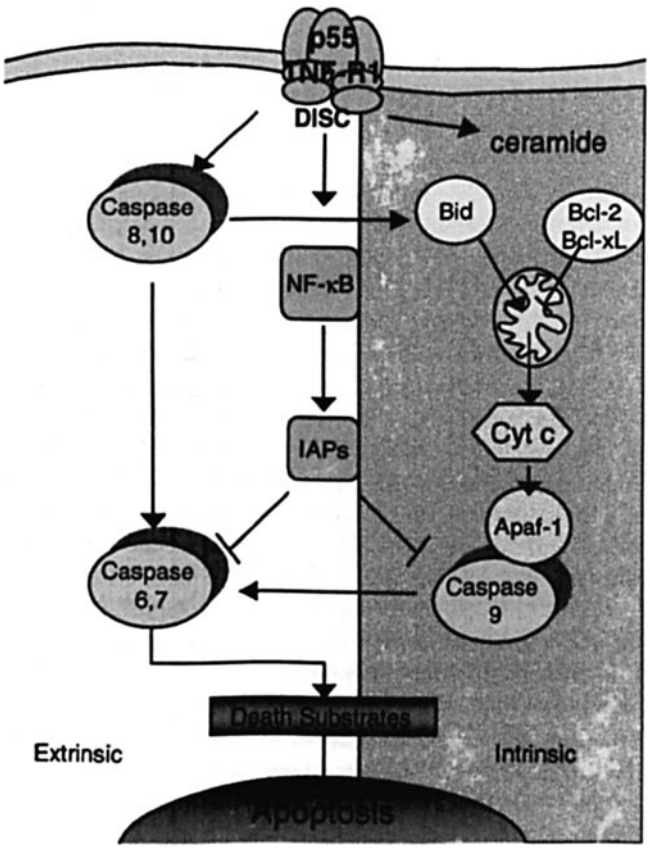

Figure 2. The extrinsic and intrinsic pathways of apoptosis. In MCF-7 cells, the intrinsic pathway is thought to be the primary means of apoptosis and is triggered by alterations in mitochondrial integrity and function independent of upstream caspase activation. In this pathway, Bcl-2 family members, such as Bcl-2 and Bcl-xL, can reduce cytochrome c release from the mitochondria and prevent apoptosis. In the extrinsic pathway, caspase-8 is activated following death receptor activation leading to direct initiation of the caspase cascade. Cross-talk can occur between the intrinsic and extrinsic pathways via caspase-8-dependent activation of Bid, which leads to further cytochrome c release. Concurrent activation of NF-κB leads to upregulation of IAPs, which can inhibit the caspases and apoptosis.

Ceramide as a Target for Chemosensitivity. Ceramide, or N-acyl-sphingosine, has been implicated in the acquired drug resistance that often characterizes breast cancer cells. It is a metabolite of sphingomyelin hydrolysis by neutral or acidic sphingomyelinases (SMase). Sphingomyelin is the most abundant lipid in the plasma membrane of mammalian cells (Fig. 3) (48). Ceramide functions as a second messenger to signaling cascades that promote differentiation, senescence, proliferation, and apoptosis. SMase activation by gamma radiation, ionizing radiation, TNF, Fas ligand, and daunorubicin leads to apoptosis. The *de novo* pathway for ceramide synthesis is also activated in response to TNF and paclitaxel agents, leading to apoptosis (49). Ceramide targets ceramide-activated protein kinase (CAPK), ceramide-activated protein phosphatases (CAPPs), and the mitochondria (50). CAPK is stimulated by ceramide through an unknown mechanism, but it has been linked to

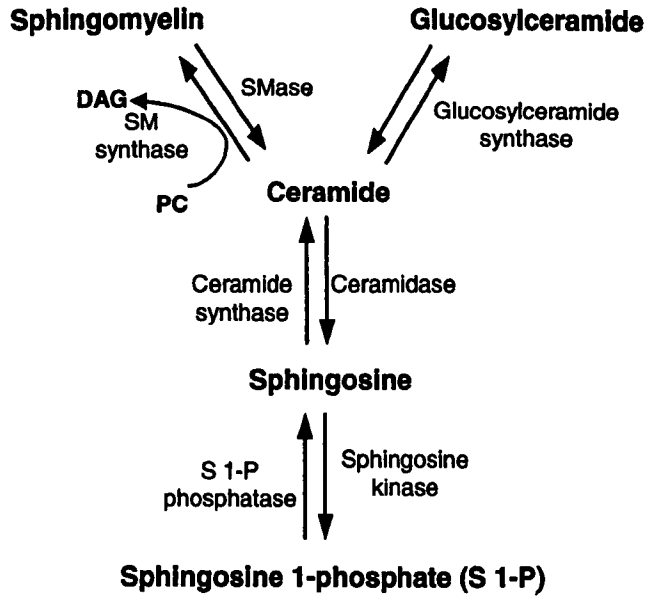

Figure 3. Ceramide metabolism. During cellular stress and apoptosis, SMase is activated to yield ceramide, which can be converted to glucosylceramide via glucosylceramide synthase. Ceramidase and sphingosine kinase activation leads to sphingosine and sphingosine 1–phosphate production whereas ceramide can be regenerated by S-1-P phosphatase and ceramide synthase activation.

interleukin-1 and TNF receptor binding and to Raf activation, which stimulates ERK. CAPPs are the best characterized of the potential ceramide targets, with possible implications for apoptosis. Generally, AKk/PKB is dephosphorylated with concomitant loss of function by the serine/threonine protein phosphatase 2A form of CAPP (49). In addition, CAPP can downregulate the antiapoptotic protein c-myc, leading to decreased protein kinase C (PKC) levels (49). The PKC family of phospholipid-dependent serine/threonine kinases plays a critical role in regulating TNF-induced cell death (51), possibly through CAPP interactions with specific PKC isoforms (15). The cytotoxic effects of chemotherapy are decreased when ceramide generation is absent or impaired and increased when degradation of ceramide is blocked (52). SMase and ceramide generation in TNF-stimulated apoptosis-sensitive MCF-7 cells does not occur in MCF-7 cells lacking p55 (TNFR1) (R-A1 cells). Following transfection of p55 (TNFR1) into R-A1 cells, TNF signaling, including NF-κB activation, was partially restored. However, the transfected cells remained resistant to TNF-induced apoptosis (53). Glucosylceramide synthase (GCS) catalyzes the glycosylation and inactivation of ceramide to glucosylceramide, which is then excreted from the cell. GCS has been shown to potentiate cellular resistance to TNF-induced apoptosis by metabolically clearing ceramide. GCS-transfected cells exhibit significantly increased GCS activity and apoptotic resistance in comparison with parent MCF-7 cells (48).

We further examined the role of ceramide in apoptosis through comparison of three MCF-7 variants. The three cell lines, M, L, and N, which were obtained from independent laboratories, differ intrinsically in their sensitivity to TNF-induced apoptosis. Continuous passaging of cells in increasing concentrations of TNF leads to decreased levels of TNF receptor and blockade of SMase activation following TNF treatment (13). The differences in the three variants may have occurred through this mechanism. The MCF-7 M-cell variant expressed low levels of p55 (TNFR-1), low levels of the proapoptotic protein Bax, and high levels of the antiapoptotic protein Bcl-2. Ceramide levels increased slightly in response to TNF treatment, but MCF-7 M cells did not undergo apoptosis. MCF-7 L cells expressed low levels of p55 (TNFR1), moderately low levels of Bax, and moderately high levels of Bcl-2. Ceramide production following TNF treatment was lower in L cells than in the M cells, and a moderate amount of apoptosis occurred after a significant delay. Decreased ceramide levels may be due to lower levels of p55 expression or impaired ability of TNF to activate SMase. MCF-7 N cells expressed high levels of p55 (TNFR1) and Bax and low levels of Bcl-2. MCF-7 N cells produced a significant increase in post-treatment ceramide levels, and a significant amount of apoptosis occurred. All three cell variants experienced a similar reduction in proliferation rate following treatment with TNF. Despite minimal expression of p55, the M and N variants maintained a weak response to TNF-induced ceramide generation. It appears that low levels of ceramide are sufficient to inhibit proliferation but insufficient to induce apoptosis. This does not account for the differential sensitivities of M and L cells to TNF, suggesting that other downstream events may be responsible for altered sensitivity to TNF-induced apoptosis (13).

Bcl-2 Family of Proteins as Anti- and Proapoptotic Effectors. The Bcl-2 family is comprised of a number of both antiapoptotic (Bcl-2, Bcl-XL, Mcl-1, Bfl-1/A1, Bcl-W, Bcl-G) and proapoptotic (Bax, Bak, Bok, Bad, Bid, Bik, Bim, Bcl-XS, Krk, Mtd, Nip3, Noxa, Bcl-B) proteins (Fig. 4) (40, 41). Antiapoptotic Bcl-2 proteins are integral membrane proteins of the mitochondria, endoplasmic reticulum, and nuclear envelope, whereas the majority of proapoptotic Bcl-2 proteins reside in the cytosol or associate with the cytoskeleton until the presence of a death signal causes them to integrate into the mitochondrial membrane (15). Expression and dimerization of Bcl-2 family proteins influence cellular sensitivity to apoptosis through the regulation of cytochrome c release, which precedes caspase activation (41). Bcl-2 proteins also interact with caspases independently of cytochrome c. The antiapoptotic protein Bcl-XL binds and inactivates the caspase adapter Apaf-1. This is balanced by proapoptotic Bcl-2 proteins that displace Bcl-XL from Apaf-1 (40). The amount of individual Bcl-2 proteins in a cell depends on several variables including the lineage of the cell, presence of activated transcription factors, the presence of nonendogenous chemicals, and estrogen responsiveness. Expression of the antiapoptotic protein Bcl-2 is increased by 17β-estradiol in estrogen-responsive MCF-7 cells (54–56). Environmental estrogens, including the pesticides o,p′ 1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane (DDT) and alachlor, are thought to

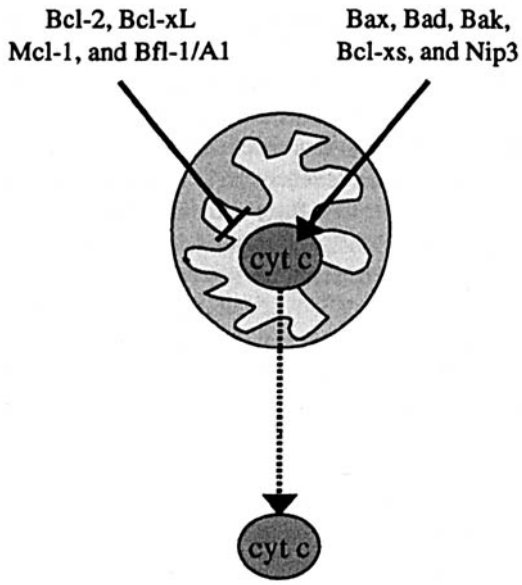

Figure 4. Bcl-2 family members Bax, Bad, Bak, Bcl-xs, and Nip3 are proapoptotic and routinely reside in the cytoplasm. When apoptosis is initiated, they can be activated and translocate to the outer mitochondria membrane where they promote release of cytochrome c. Antiapoptotic Bcl-2, Bcl-xL, Mcl-1, and Bfl-1/A1 are integral membrane proteins. They inactivate proapoptotic Bcl-2 family members and block cytochrome c release. (Note: only Bcl-2 family members identified in MCF-7 cells are shown.)

mimic this effect. They have been shown to suppress TNF-induced apoptosis in MCF-7 cells but not in ER-negative cells (55). Our laboratory found that high levels of Bcl-2 and low levels of Bax correlate with decreased protease activity in MCF-7 variants (13). However, as is the case with ceramide, this does not entirely account for the intrinsic apoptotic resistance of MCF-7 M cells.

Chemotherapeutic drugs exert their effect in part by modulating the expression of several members of the Bcl-2 family in MCF-7 cells. Paclitaxel and thiotepa upregulate several proapoptotic Bcl-2 proteins and downregulate antiapoptotic Bcl-2 proteins (57). Similar results are seen with doxorubicin, which causes a decrease in Bcl-2 expression and increase in Bax expression (58). Etoposide and 4-hydroperoxycyclophosphamide both increase proapoptotic Bax levels (58, 59) and, when used in combination, reduce Bcl-2 expression and increase Bax levels more than either agent alone (59). Docetaxel sensitizes MCF-7 cells to chemotherapy-induced apoptosis, presumably through its ability to phosphorylate and inactivate Bcl-2 (60). However, chemotherapy cannot always overcome the effects of the proapoptotic Bcl-2 proteins. Bcl-2 and Bcl-X_S (61) expression have been correlated with resistance and poor response to chemotherapy in many cell types, including MCF-7 cells (62). Plasmid introduction of anti-Bcl-2 antibody gene (anti-Bcl-2 sFv) enhances chemotherapy-induced block of Bcl-2 in MCF-7 cells and represents a novel cancer treatment (62). In addition, a novel Bcl-2/Bcl-XL-bispecific antisense oligonucleotide has demonstrated remarkable efficacy in MCF-7 cells (63). Clinically, increased levels of Bax are correlated with a good response to chemo- and radiotherapy, whereas increased levels of Bcl-2 and Bcl-XL are correlated with a poor response (64–66).

PI3K/Akt Is an Important Determinant of Chemoresistance. Akt, also known as protein kinase B (PKB), is activated by a variety of stimuli, including hormones and growth factors. Insulin-like growth factor-I (IGF-I) is a well-known mitogen in breast cancer that activates the MAPK pathway and the PKB/Akt pathway (67). MCF-7 cells have been shown to initiate DNA synthesis in response to IGF-I exposure (68). The events and molecules involved in Akt signaling pathways are not fully known; however, it is known that Akt activation occurs downstream of phosphoinositide-3 kinase (PI3K) (69–71), a kinase that is also upstream of several antiapoptotic protein kinase Cs (PKC) (72). PI3K activates lipid second messengers that are essential for the translocation of Akt to the plasma membrane, where it is phosphorylated and activated by phosphoinositide-dependent kinase-1. Akt phosphorylates and regulates proteins involved with several cellular functions, including apoptosis (Fig. 5) (70). Dysregulation of the Akt signaling pathway and amplification of the Akt gene are thought to promote cell proliferation and survival (70). Among the proteins that Akt phosphorylates are Bad and NF-κB (24, 72). Phosphorylation inactivates the proapoptotic protein Bad by causing it to be sequestered in the cytosol by 14-3-3 proteins (40). Raf/Erk, a component of the p21ras pathway (one of several Ras oncogene pathways implicated in cancer) (71), and Akt act in cooperation to

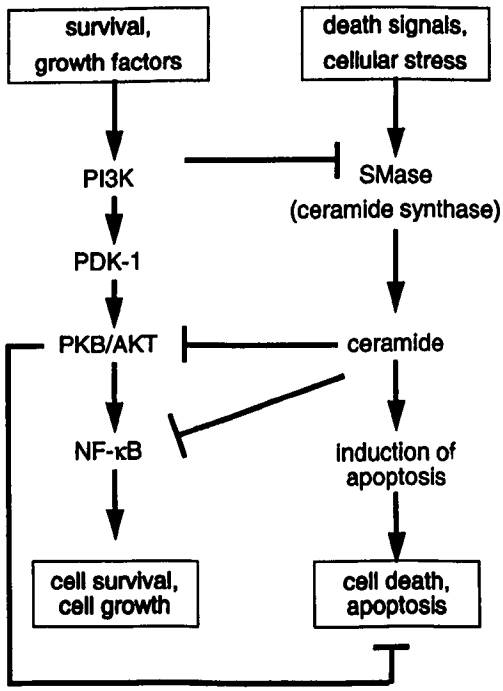

Figure 5. Survival and death signals proposed to be important in MCF-7 cells. The PI3K/Akt-NF-κB pathway is the most important survival pathway identified to date in MCF-7 cells. Its expression can impact apoptosis and thereby the efficacy of chemotherapeutic drugs. The ceramide pathway plays a key role in promoting apoptotic signaling events in MCF-7 cells.

induce p65 NF-κB activity (70). Our laboratory has found that in MCF-7 cells, PI3K, and Akt suppressed a dose-dependent induction of apoptosis by TNF-α and that PI3K and Akt stimulated NF-κB activation in a dose-dependent manner, suggesting a common link between these two pathways. IκB is able to block this Akt-NF-κB cross-talk (73). Polyphosphate inhibitors of the PI3K/Akt pathway, which target Akt, increase sensitivity to apoptosis (70). A recent study proposes a new model for antiestrogen resistance that requires the activation of ERα by PI3K and Akt in the absence of estrogen (9). In addition, cross-talk between the PI3K/Akt/NF-κB pathway and ceramide appears to mediate cell survival–cell death decisions (74, 75).

NF-κB Transcriptional Activity Is Driven by PI3K/Akt. NF-κB is a multisubunit nuclear transcription factor that regulates several cellular functions, including cell growth, differentiation, development, adaptive responses to redox balance, and apoptosis. In its inactive form, NF-κB resides in the cytoplasm bound to inhibitory IκB proteins that shield the DNA binding site. External stimuli, including proinflammatory agents, infectious agents, stress, and chemotherapeutic drugs, activate NF-κB by phosphorylation and subsequent degradation of IκB proteins, whereupon NF-κB is transported into the nucleus and transcription of the target genes occurs. NF-κB is required for the induction of more than 150 genes (76). Whether NF-κB targets pro- or antiapoptotic genes depends on the stimulus-specific signaling pathway activated. For instance, the TNF-activated death domain kinase RIP induces proapoptotic NF-κB activity and this activity can be blocked by caspase-8 cleavage of RIP (77). This relationship represents a regulatory mechanism that balances pro- and antiapoptotic signals. Antiapoptotic target genes of NF-κB include the IAP family of caspase inhibitory proteins, TRAF1 and TRAF2, which are thought to suppress caspase-8 activation, Bfl1/A1, Bcl-XL, and inducible nitric oxide synthetase. Interestingly, a recent report has also shown the ability of an NF-κB pathway to mediate suppression of Bax expression (78). Proapoptotic targets are the promoters of death receptors and ligands (e.g., Fas, FasL, and TRAIL receptor). NF-κB is overexpressed in ER-negative MCF-7 cells and other breast cancer lines and has been shown to play a role in susceptibility to apoptosis to several chemotherapeutic drugs in our MCF-7 cell variants (79). MCF-7 cell variants resistant to the antiestrogen ICI 182,780 exhibit upregulation of cyclic adenosine monophosphate response element and NF-κB binding. In addition, parthenolide, a potent and specific inhibitor of NF-κB, inhibits the anchorage-dependent proliferation of antiestrogen-resistant but not antiestrogen-responsive cells, implying an increased reliance on NF-κB signaling for proliferation in cells that have survived prolonged exposure to ICI 182,780 (80).

Conclusions and Suggested Future Research

MCF-7 cell variants are a novel tool for the study of breast cancer resistance to chemotherapy, because they appear to mirror the heterogeneity of tumor cells *in vivo*. As these cells are exposed to "selective" pressures in their environment (i.e., long-term exposure to TNF or other chemotherapeutic drugs), they become drug resistant. Numerous studies have helped to partially elucidate the mechanisms behind the acquisition of chemoresistance. Several potential targets for reversing resistance have been identified, such as caspase-3 upregulation, cross-talk between the intrinsic and extrinsic caspase cascades, upregulation of ceramide, inhibition of GCS, and antagonism of Bcl-2 and the PI3K/Akt/NF-κB pathways. Selective manipulation of these signal transduction pathways in combination with current chemotherapeutic drugs may lead to an increased potency and efficacy of these agents. Recent gene array studies conducted in our laboratory have identified some additional candidate antiapoptotic proteins, such as the member of the MAPK family, MEK5-BMK/Erk5, which may contribute to antiapoptotic signaling associated with MCF-7 M cell intrinsic apoptotic resistance (81). Additional studies will capitalize upon clues discerned from gene array data, which will no doubt suggest a number of new potential therapeutic targets for breast cancer.

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