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## Mitochondria: gatekeepers of response to chemotherapy

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### Abstract

Mitochondria are cellular organelles that regulate commitment and execution of apoptosis. The intrinsic apoptotic pathway culminates in the permeabilization of the mitochondrial outer membrane and dismantling of the cell. Apoptosis of cancer cells is a favorable outcome when administering chemotherapeutic treatment yet the basis for why some cancers are sensitive to chemotherapy while others are not has historically been poorly understood. In this review, we present recent work that has demonstrated the importance of mitochondrial apoptotic priming, or how close a cell is to the threshold of apoptosis, in determining whether a cell will undergo apoptosis after chemotherapy treatment. Differential levels of apoptotic priming in tumors create bona fide opportunities and challenges for effective use of targeted and cytotoxic chemotherapies.

### Keywords

apoptosis; chemotherapy; mitochondrial apoptotic priming; chemotherapeutic window

### Why does chemotherapy work?

A longstanding question among not only patients but also the oncologists that are treating them is “why does chemotherapy work?” This important question is asked because chemotherapy does, at times, work impressively well, leading to long-term cures of otherwise fatal neoplasms. However, despite a growing understanding of how cancers arise, grow, metastasize and eventually overcome the host, the mechanisms behind successful treatment of cancers are poorly understood[1,2]. The key determinants of response to chemotherapy will be explored in this review, with a focus on how the pathway of mitochondrial apoptosis affects treatment outcome.

### Chemotherapy and apoptosis

Treatment of human malignancies with chemotherapy with curative intent has been successfully carried out for over 50 years with millions of cancer survivors enjoying long lives after treatment[3]. However, millions more have succumbed to their disease. Regardless of whether they are considered “cytotoxic” or “targeted,” most chemotherapies function by inducing a form of irreversible programmed cell death called apoptosis[4–6].

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Apoptosis can proceed via two distinct pathways: intrinsic and extrinsic[7]. The extrinsic apoptotic pathway is engaged upon activation of cell surface death receptors and has a limited, controversial role in chemotherapy-induced apoptosis[2,4,6]. We will therefore focus solely on the intrinsic, or mitochondrial apoptotic pathway in this review.

Mitochondrial apoptosis is controlled by the pro- and anti-apoptotic proteins of the BCL-2 family which can be divided into three categories based on their intracellular function and sequence homology (reviewed in [8]). One category includes the anti-apoptotic proteins BCL-2, BCL-w, MCL-1, BFL-1, and BCL-X<sub>L</sub> which contain all four BCL-2 homology domains (BH1-4). These proteins prevent apoptosis by binding and sequestering their pro-apoptotic counterparts. The second category, the BH3-only proteins, includes the pro-apoptotic proteins PUMA, BIM, BID, BAD, BIK, NOXA, and BMF, which contain only the BH3 domain. The final category, the effectors, contains BAX and BAK which contain domains BH1-3 and can be activated by a subset of BH3-only proteins which include BIM, BID and, potentially, PUMA[9–15].

Activation of BAX or BAK initiates a series of steps that results in commitment to apoptotic cell death. It had been suggested that BAX and BAK are activated not by BIM and BID but instead via the inactivation of anti-apoptotic BCL-2 family members[16]. However, recent studies have confirmed the direct binding and activation of BAX by BIM and BAK by BID[9,13,17]. Upon activation, BAK and BAX oligomerize and directly cause mitochondrial outer membrane permeabilization (MOMP), a critical event during apoptosis. Cytochrome c and other factors are released after MOMP and associate with several cytosolic proteins including APAF-1 to activate caspases for dismantling of the cell[7]. Even in the absence of caspase activation, post-MOMP mitochondria are progressively impaired in their ability to generate ATP and cannot maintain cellular survival except in specific, non-physiological circumstances[7,18,19]. MOMP can thus be considered the “point of no return” in mitochondrial apoptosis.

MOMP, and consequent apoptosis, is triggered clinically when a chemotherapeutic agent induces a sufficient amount of stress within a cancer cell by damaging critical cellular components such as microtubules, DNA, or key signaling pathways[2,4,6]. The cell responds by unleashing pro-apoptotic proteins or downregulating anti-apoptotic members of the BCL-2 family, either of which can lead to a shift in the life/death balance in the cell irreversibly toward destruction[4,6]. Based on this understanding, both the state of the BCL-2 family of apoptosis-regulating proteins in the cell prior to encountering the stress as well as the magnitude of the dynamic response to the stress can have a profound impact on the eventual fate of the cell. These concepts will be explored further below.

## Measuring mitochondrial apoptotic priming

Regulation of the balance of pro- and anti-apoptotic proteins within cells, and thus how close a cell is to the threshold of apoptosis, is dependent on many factors. In order to avoid apoptosis, a cell must express a sufficient amount of anti-apoptotic proteins to bind and inactivate what pro-apoptotic counterparts are also present. Furthermore, most cells contain an additional amount of “buffering” anti-apoptotic proteins that can inactivate further pro-death signals that are encountered on a stochastic basis under normal, physiological conditions[5]. The amount of buffering that a cell may employ varies by the type of cell: many cells in the hematopoietic and immune systems maintain relatively small buffers of anti-apoptotic proteins[5], likely to enable their quick and efficient elimination as the need arises[20]. Conversely, cells with a longer lifespan such as the highly specialized, fully differentiated cells that make up vital organs are more buffered against short-term, stochastic fluctuations in cellular stress[5] that could erroneously trigger cell death in a vital cell to the

detriment of the host's survival (reviewed in [21]). The specific factors that control the level of anti-apoptotic buffering within healthy cells are unknown but may involve the epigenetic programming that maintains cell identity[22].

The varying levels of anti-apoptotic buffering present within cells can affect the outcome of any event that stresses a cell, including chemotherapy treatment. For instance, a cell may express a very large reserve of unbound anti-apoptotic proteins, which are available for binding of their pro-apoptotic counterparts, and therefore be protected against even a substantial amount of subsequent pro-death signaling. Alternatively, a cell may express only enough unbound anti-apoptotic proteins to just barely keep the pro-apoptotic proteins in check and would therefore not be protected from even a small stressor. The cell that has a small reserve of unbound anti-apoptotic proteins is "primed" for apoptosis while the cell that has a large reserve is "unprimed" (Figure 1). Another way to illustrate this dichotomy is to envision two cells, one being close to the threshold (cliff's edge) at which apoptosis is triggered while the other is far from that same threshold. The one closer to the edge is considered primed while the other is unprimed. When these two cells are treated with equal doses of chemotherapy (pushed toward the cliff's edge), the primed cell will be more likely to trigger apoptosis than the unprimed cell if the dynamic response to chemotherapy (strength of push toward edge) is equal (Figure 2A). While it aids understanding to define priming this way, the actual measurement of priming is done by a functional test and thus a label of "primed" and "unprimed" can only be assigned based on a given cell's response to systematic pro-apoptotic stimuli as described below.

Because there may be substantial utility in knowing how close a cancer cell is to the threshold of apoptosis, an assay called "BH3 profiling" has been developed to measure priming in cancerous and normal cells. The basic principle is to expose mitochondria to peptides derived from the BH3 domains of pro-death BH3-only proteins, and measure the resulting magnitude of mitochondrial outer membrane permeabilization (for full methods see [23]). Heightened sensitivity of mitochondria to BH3 peptides, such as BAD BH3 or NOXA BH3, with selective binding to anti-apoptotic proteins might indicate selective dependence on BCL-2 or MCL-1[24–26]. Heightened sensitivity to peptides that exhibit promiscuous binding to anti-apoptotic proteins, such as BIM BH3 or PUMA BH3, indicates a highly primed state, with a low reserve of unbound anti-apoptotic proteins[5,26–28].

In practice, the assay is typically performed by gently permeabilizing the plasma membrane of cells and adding fixed doses of BH3 peptides derived from the BH3 domain of pro-apoptotic BH3-only proteins. Mitochondrial potential is then monitored for detection of MOMP which occurs when the anti-apoptotic reserve is exhausted within a cell and BAX and/or BAK are activated (Figure 1). Cells can be labeled as primed or unprimed based on the extent of mitochondrial depolarization (MOMP) that occurs in response to a fixed titration of pro-death BH3 peptides – the faster that a cell is depolarized, or the lower concentration of peptide required for MOMP, the more primed it is.

Functional assays, such as BH3 profiling, are critical for measurement of apoptotic priming within a cell. The activities of the BCL-2 family of proteins is regulated by a plethora of post-translational modifications and interactions with other proteins[29]. Measuring the expression level of each member of the BCL-2 family, their modifications and the proteins they are interacting with is not a practical task and, due to additional unknown factors that may modify their activity, may not even provide an accurate assessment of priming and thus creates a need for a function-based assay. By treating a cell with fixed doses of pro-apoptotic signaling in BH3 profiling, it is possible to measure the integrated functional output of the BCL-2 family efficiently and meaningfully[5].

## Mitochondrial apoptotic priming determines tumor response to cytotoxic chemotherapy

Using BH3 profiling to measure levels of apoptotic priming across a range of cancer types, it has been shown that primed cells readily undergo apoptosis in response to cytotoxic chemotherapy treatment while unprimed cells are less likely to do so (Table 1)[5]. Notably, this holds true not only in cancer cell lines but also primary tumors and can be predictive of how patients will respond to chemotherapy in the clinic[5,28]. In addition, cells within tumors that have undergone treatment and then recurred are frequently less primed, making them less sensitive to subsequent rounds of chemotherapy[5,28]. The selective pressure of chemotherapy treatment likely culls primed cancer cells, leaving only unprimed cells to repopulate the tumor[5,28]. These unprimed relapsed tumors are frequently intractable.

Experiments measuring mitochondrial priming have also uncovered a greater understanding of apoptotic signaling in cancer cells and normal cells. A longstanding misconception is that cancers must “disrupt” or “disable” apoptosis[4,30,31], which some interpret as meaning that cancer cells are inherently less sensitive to subsequent apoptotic signaling than normal cells. A “disruption” or “disabling” of apoptosis would require a complete loss of signaling in the mitochondrial apoptotic pathway via, for example, a total loss of BAX and BAK expression, yet this is rarely observed in cancers. Cancers commonly upregulate anti-apoptotic proteins or downregulate pro-apoptotic counterparts to keep apoptosis at bay (reviewed in [29,32] but this does not equate to a disruption or disabling of the apoptotic pathway. In fact, there is little evidence that cancer cells are more refractory to apoptosis than normal cells. While most cancers have an intact apoptotic pathway, some tumors are even highly primed for apoptosis as demonstrated by their efficient mitochondrial depolarization in response to pro-apoptotic signaling in the form of BH3 peptides. This depolarization often exceeds that observed in normal somatic cells[5]. This confirms that most cancers not only express the critical effectors BAX and/or BAK and have an intact mitochondrial apoptotic pathway but are also primed for apoptosis. The cancers that are very primed are those that respond most favorably to chemotherapy (acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia) while those that are unprimed respond poorly (endometrial and renal cell carcinomas, serous borderline tumors)[5,27,28]. The therapeutic index of conventional chemotherapy, which is directed against ubiquitous targets such as DNA and microtubules, probably derives largely from the fact that cancer cells are actually more ready to engage the machinery of apoptosis than normal cells, not less[5,28]. In fact, it is unlikely that anyone would ever be cured via conventional chemotherapy, if some cancer cells were not sometimes much more primed for apoptosis than most normal cells.

Other measures of the readiness of cancer cell mitochondria to undergo cell death have been utilized to predict cellular responses to chemotherapy. For example, measuring the propensity of tumor cells to spontaneously activate caspase 3 has prognostic value for response to chemotherapy in anaplastic lymphoma[33]. In colorectal cancer, measuring and integrating the protein expression levels of several BCL-2 family members may predict how patients will respond to chemotherapy[34]. Although these other measurements are not functional tests of apoptotic priming as defined here, they nonetheless validate the importance of pretreatment apoptotic signaling in cell fate decisions.

### What determines how primed a tumor will be?

Although cancers have wide ranging levels of mitochondrial priming which contribute to their responses to chemotherapeutic agents[5,27,28], it is unclear what determines the level of priming within a cell or tumor. At the cellular level, an attractive hypothesis is that the

level of priming evident in a tumor cell is determined, at least in part, by the level of priming evident in the cell that gave rise to the tumor itself. Lending evidence to this concept is the observation that the most highly primed cancers are derived from the most highly primed normal tissues, and vice versa. For instance, acute lymphoblastic leukemia cells are among the most highly primed and the most chemosensitive of cancers. They derive from the lymphocytic lineage, which is among the most highly primed lineages in the body[5,25,28] and is among the most chemosensitive[35]. Conversely, chemoresistant primary tumors, including renal cell carcinomas and endometrial cancers, are unprimed, as are the normal, healthy cells they are derived from[5]. Thus, cell lineage may be a strong determinant of the overall apoptotic priming of a tumor.

At the molecular level, the level of priming is regulated by the expression and post-translational modification state of BCL-2 family proteins – but what in turn regulates this? Most likely, priming results from the combined contributions of many signaling pathways. In normal cells, various signaling pathways can modulate BCL-2 family expression for regulation of survival. For example, the survival of mature B cells requires pro-survival B cell receptor signaling[36,37] which was recently shown to be mediated by PI3 Kinase (PI3K)[38]. The PI3K prosurvival signals in these cells are carried out by Akt and the FOXO family of transcription factors that eventually modulate levels of the pro-apoptotic protein BIM[38].

The same pathways that modulate apoptosis in healthy cells are aberrantly expressed in cancer cells to maintain survival. PI3K, for example, is inappropriately activated in a wide range of malignancies and confers a strong enough survival advantage to cancerous cells that it is being targeted pharmacologically for cancer therapy (reviewed in [39,40]). Other common oncogenes and tumor suppressors that are deregulated in cancer have been shown to have pro- or anti-apoptotic effects including p53[41], Notch[42] and Wnt[43] among others including, of course, the BCL-2 family. Interestingly, activation of some oncogenes, especially c-Myc, can have a strong pro-apoptotic effect[44–46] and thus likely contribute to the final overall level of priming in cancer cells.

Not surprisingly, the tumor microenvironment is also a strong modulator of apoptotic priming. Chronic lymphocytic leukemia (CLL), for example, is commonly treated with chemotherapy yet cancerous cells exhibit differing levels of chemosensitivity, with a subpopulation of cells proving to be intractable and supporting relapse[27,47]. Cells residing within lymph nodes upregulate anti-apoptotic BCL-X<sub>L</sub> and BCL2A1 (BFL-1) to reduce apoptotic priming, making them more likely to survive therapy treatment[27,47]. The interactions of cancer cells with stroma can lead to chemotherapy resistance in both solid and liquid tumors (reviewed in [48]). In addition, stimulation with exogenous growth factors[46,49,50] can affect BCL-2 family protein expression and sensitivity to chemotherapy. In one such example, hepatocyte growth factor (HGF) was recently identified in a cancer cell and stroma co-culture screen to induce a chemoresistant phenotype in tumor cells by activating PI3K[50], whose role in modulation of apoptosis has already been discussed.

The pretreatment level of priming in a tumor is likely determined by the level of priming in the cell of origin and modulated by the microenvironment of the cell and by the pro- and anti-apoptotic effects of the various perturbations that have occurred during tumorigenesis.

## The chemotherapeutic window

Understanding the mitochondrial priming of the tumor alone, however, is insufficient to understanding the source of a chemotherapeutic window. The key to achieving a desirable outcome from chemotherapy treatment is as much dependent on the lack of priming in vital

organs as the priming in the tumor. When comparing apoptotic priming across healthy tissues, one can see that most healthy tissues are unprimed which allows vital organs such as the liver, heart, brain and kidneys to survive high doses of chemotherapy relatively unscathed[5]. The lack of priming in these vital tissues is demonstrated by their attenuated response to relatively large doses of pro-apoptotic BH3 peptides[5]. However, the molecular mechanisms that maintain this low level of priming in vital tissues are unknown and an active area of investigation. There are, of course, some effects of chemotherapy on these tissues including long term pathologies, such as doxorubicin-induced cardiotoxicity[51], but these organs generally continue to function during and after chemotherapy treatment. Some healthy tissues, however, exhibit a high level of apoptotic priming, especially the hematopoietic system, which renders them sensitive to chemotherapy. A loss of the bulk of the hematopoietic system seems to be entirely survivable, though, as evidenced by the frequent myelosuppression that accompanies chemotherapy treatment even in those patients that emerge with cures. In general, cells from tumors that are chemosensitive are more primed than their non-transformed counterparts, thus creating a therapeutic window for tumor response to therapy[5]. For the most broadly cytotoxic chemotherapies that target common cellular elements across all cells, successful treatment relies on a level of priming in the tumor higher than other healthy tissues (Figure 3).

### Mitochondria in targeted chemotherapies and immunochemotherapies

Mitochondrial priming has a likely role in cellular response to “targeted” chemotherapies as well. Although “classical” cytotoxic chemotherapies can have quite specific targets, for example, paclitaxel for microtubules and topotecan for topoisomerase II, for the purpose of this review only agents that target non-ubiquitous cellular components will be considered targeted agents. Much progress has been recently made in the development of targeted agents that have the potential to be selectively toxic to cancer cells that are dependent on that target over healthy tissues that lack it (reviewed in [52]). Most of these targeted agents exert their anti-cancer effects by engaging the same mitochondrial apoptotic pathways as classical chemotherapies which proposes a potential role of apoptotic priming in the response to these agents as well[53–55]. The largest determinant of apoptotic response to targeted agents, of course, is a given cell’s dependence on the protein or signaling pathway being targeted (Figure 2B and Table 1). For example, a cell that is dependent on EGFR will upregulate pro-apoptotic BIM when EGFR signaling is inhibited, thus pushing the balance of BCL-2 family proteins toward apoptosis[55–57]. Cells lacking dependence on EGFR are unaffected by these inhibitors[55–57], and thus even a high level of mitochondrial priming in these cells is unlikely to trigger cell death (Figure 2B). Conversely, cells that are not highly primed but exhibit a strong dependence on the targeted pathway may respond to the inhibitor with enough pro-apoptotic stress signaling that apoptosis is triggered (Figure 2B). Therefore, the magnitude of the response to the inhibitor is likely the most important factor in determining whether the cell will live or die. However, in cells that exhibit a similar level of dependence on a targeted pathway and thus a similar dynamic signaling response to the inhibition of that pathway, the level of mitochondrial priming may affect whether apoptosis will occur. A highly primed cell would need a smaller dynamic pro-apoptotic response to an agent to trigger apoptosis than an unprimed cell (Figure 2C and Table 1).

It is likely that using targeted agents in cells that are dependent on the target yet are unprimed may have clinical benefits even without apoptosis being induced by the targeted therapy alone. Specifically, agents targeting cancer-specific proteins or growth signaling pathways may induce a level of apoptotic stress within a cancer cell that is perhaps not sufficient to trigger apoptosis but may upregulate pro-apoptotic proteins, thus increasing mitochondrial apoptotic priming. In this context, an additional chemotherapeutic agent, targeted or not, could provide the final bolus of pro-apoptotic signaling necessary to activate

MOMP (Figure 2D). This has been convincingly demonstrated with many “priming” agents[27,54,58–60]. These priming agents, with diverse targets including histone deacetylases and PI3K, modify levels of the BCL-2 family to improve responses to cytotoxic chemotherapy[54,59]. In addition, a novel class of agents, BH3 mimetics, have been shown to potently and specifically inhibit several anti-apoptotic members of the BCL-2 family including BCL-2, BCL-X<sub>L</sub> and BCL-W. The administration of these agents can increase the priming of a cancer cell and thus make other, concurrently-administered chemotherapies, more effective[28,34,61–64].

Mitochondrial priming likely plays a smaller role in the responses of cancer cells to therapies that are based on activating an antitumor response by the immune system (Table 1). These therapies include monoclonal antibody therapies directed at cell surface markers expressed on tumor cells, immuno-modulatory agents, and allogeneic stem cell transplantation. Although monoclonal antibodies targeting tumor cells can sometimes directly induce apoptosis in target cells, they most commonly rely on antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity to activate immune-effector cells for maximal effectiveness[65]. In addition, agents that modulate the immune system directly, such as the anti-CTLA4 antibody (ipilimumab), enhance the antitumor responses of cytotoxic T cells[66]. Because the mitochondrial apoptotic pathway is involved in the direct anti-tumor cell activity of some of these therapies, priming may have a role in determining response. However, this role would likely be overshadowed by other key determinants of response and resistance including the presence or absence of the cell surface marker on the tumor cell and immune competence [65,67]. In the case of allogeneic stem cell transplant, an adaptive immune response of the donor’s T-cells against the host tumor is required. At least in acute myelogenous leukemia, success of this strategy is not affected by the apoptotic priming of tumor mitochondria[28].

### Alternative determinants of chemotherapy effectiveness

Although recent results have suggested that priming is the major determinant of chemotherapy effectiveness[5,27,28], several laboratories have provided evidence of some alternatives. In mitochondria specifically, chemotherapy response has been linked to defects in mitochondrial respiratory chain complexes caused by loss of specific cytochrome c oxidase subunits[68]. The loss of these subunits was observed clinically in a subset of oesophageal adenocarcinomas and was associated with increased sensitivity to chemotherapy, suggesting that mitochondrial defects can develop during tumorigenesis and may contribute to the chemotherapeutic window.

A longstanding theory of chemotherapy effectiveness has been that the proliferation rates of cancer cells determines their sensitivity to chemotherapy. However, this explanation is inadequate since clinical observations have shown that many chemosensitive cancers proliferate very slowly while many chemoresistant cancers proliferate quickly[69–73]. In addition, some non-proliferating healthy cells (resting B cells for example) are extremely sensitive to DNA damaging agents[35], thus making it less likely that proliferation alone is the basis for chemosensitivity, despite its favored place in textbooks and general opinion in oncology.

### Concluding remarks

Mitochondria have a well-established and prominent role in chemotherapy effectiveness that should be exploited for cancer therapy. Specifically targeting the BCL-2 family is a strategy that has already shown promise: the BCL-2/BCL-X<sub>L</sub>/BCL-w inhibitors ABT-737 and its derivatives have activity against multiple types of blood cancers[62] and some solid tumors[74–76]. Other strategies to inhibit these proteins are also in various stages of

preclinical development (reviewed in [77]). One anti-apoptotic member of the BCL-2 family, MCL-1, is also a promising target in cancer therapy but has, so far, eluded efforts to effectively target it for cancer therapy. Other strategies to target this family, including stapled  $\alpha$ -helices that can penetrate cells and act as inhibitors of anti-apoptotic proteins, are also being pursued [78,79]. Another interesting strategy to overcome chemotherapy resistance is the direct pharmacological activation of the pro-apoptotic effector BAX [80].

The successful use of chemotherapy to treat malignancies for over five decades is based, at least in part, on high levels of mitochondrial priming in chemosensitive tumors, a concept only recently demonstrated [5]. As we continue to broaden our understanding of apoptosis and how it is regulated, we hope to uncover critical new vulnerabilities within this pathway in cancers.

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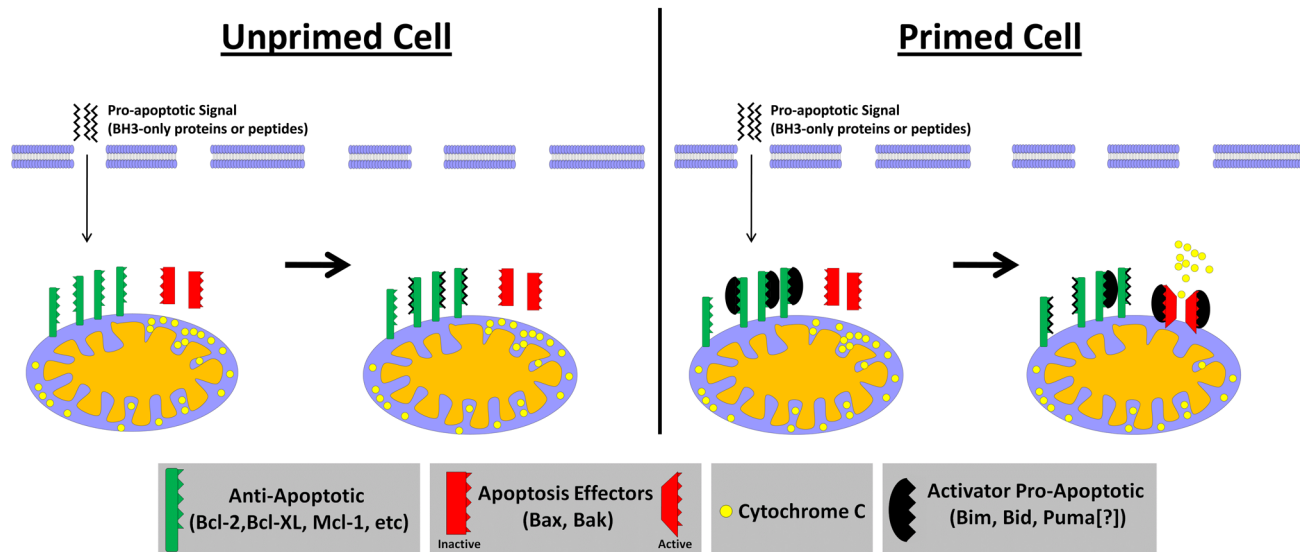
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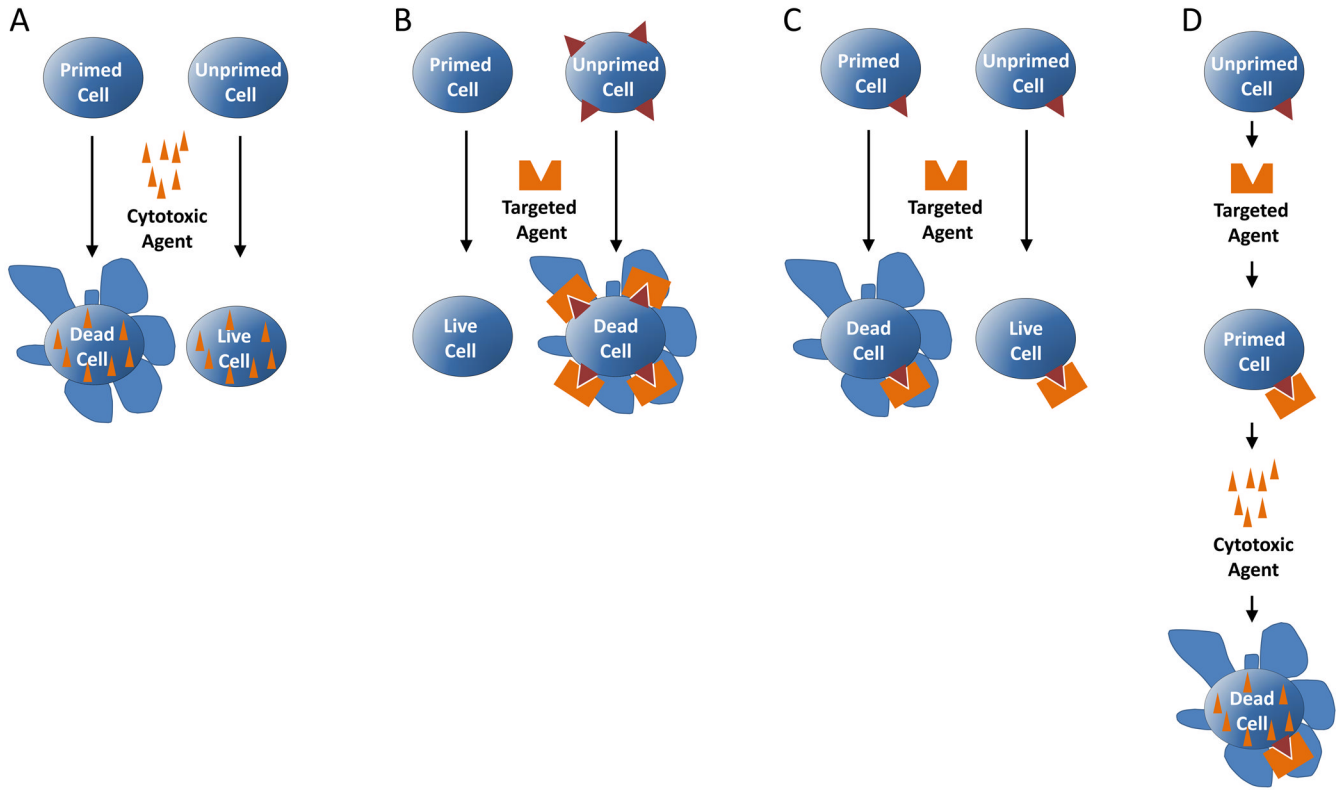
**Priming can affect cell fate decisions:**



**Figure 1. A model for how pretreatment mitochondrial priming can affect response to pro-death signal**

In unprimed cells, exogenous pro-apoptotic signals are bound and sequestered by unbound anti-apoptotic proteins. In primed cells, pro-apoptotic signals displace the BH3-only proteins bound to anti-apoptotic proteins which are then able to activate BAX/BAK.

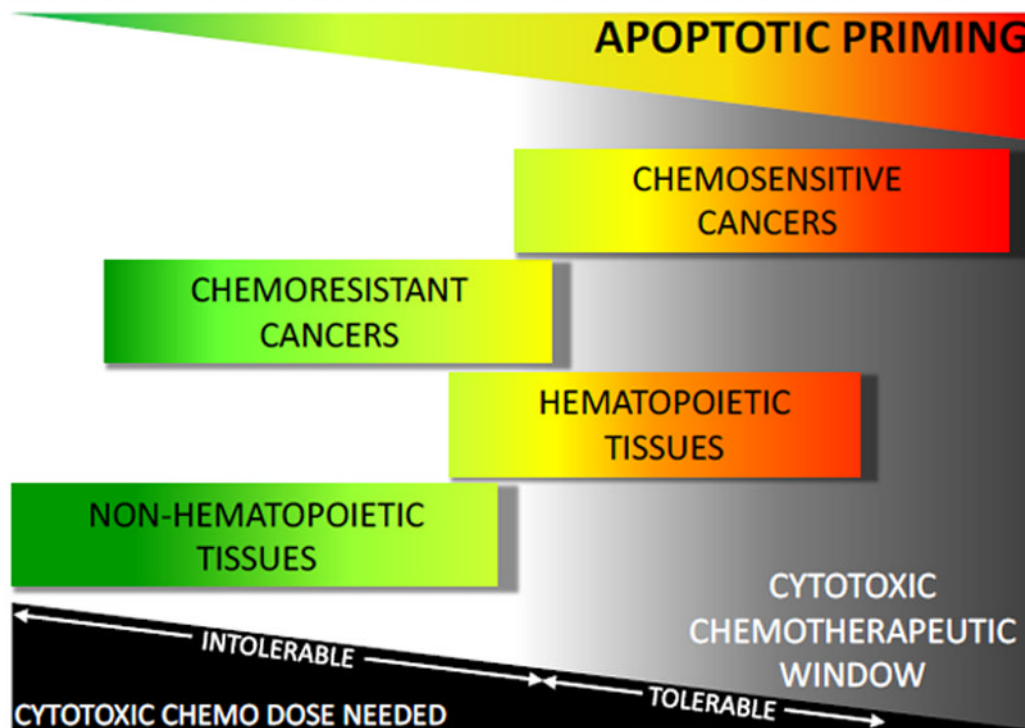
### Apoptotic priming can affect responses to therapies:



**Figure 2. Apoptotic priming can affect response to chemotherapy**

(A) A cell that is primed for apoptosis is more likely to undergo cell death in response to chemotherapy than an unprimed cell. (B) A cell that is primed for apoptosis nonetheless does not undergo cell death in response to a targeted agent if it does not exhibit a dependence on the target of the therapy. A cell that is unprimed, but exhibits a strong dependence on the target of the therapy may unleash sufficient pro-apoptotic signaling to trigger apoptosis. (C) Two cells that exhibit similar dependence on the target of the therapy may have different treatment outcomes based on levels of apoptotic priming present in cells prior to therapy. (D) A cell that is unprimed yet exhibits dependence on the target of the therapy may become primed in response to the therapy if apoptosis is not triggered directly by the single agent. An additional bolus of pro-apoptotic signaling, here provided by treatment with a cytotoxic chemotherapy which would not kill an unprimed cell, is sufficient to trigger apoptosis.

**The chemotherapeutic window is based on apoptotic priming:**



**Figure 3.** Apoptotic priming varies between tumors and healthy tissues and provides a window for chemotherapeutic efficacy.

**Table 1**  
Major determinants of chemotherapy treatment success (why chemotherapy works):

Drug Class	Targets	Examples	Determinants of Therapy Success		
			Primary	Secondary	Cancer Type
Classical cytotoxic therapy	Ubiquitous cellular elements (microtubules, DNA, topoisomerases)	Paclitaxel (microtubules), Topotecan (topoisomerase II), Doxorubicin (DNA)	Pretreatment mitochondrial apoptotic priming	Signaling dynamics post treatment	Multiple myeloma, ALL, ovarian cancer[5]; CLL[27]; AML[28]
Targeted therapy	Cell-specific targets (mutated oncogenes, differentially expressed oncogenes)	Erlotinib (EGFR), Vemurafenib (V600E), Imatinib (BCR-ABL)	Presence or absence of target	Pretreatment mitochondrial apoptotic priming, signaling dynamics post treatment	Non-small-cell lung cancer[81,82]; melanoma[83]; CML[84]
Antibody-based therapy	Cell surface markers	Rituximab (anti-CD20 antibody), brentuximab-vedotin (anti-CD30:MMAE antibody-drug conjugate)	Presence or absence of target	Immune system competence	Lymphoma[85]; lymphoma and Hodgkin's disease[86]
Immune modulation (reversal of immune checkpoint blockade)	Immune system (tumor tolerance, tumor-associated macrophages)	Ipilimumab (anti-CTLA4 antibody),	Immune system anti-tumor response	Immune system competence	Melanoma[66,87]