Apoptosis signaling to mitochondria by death receptors and DNA damaging anti-cancer regimens
Werner, A.B.

Citation for published version (APA):
Werner, A. B. (2004). Apoptosis signaling to mitochondria by death receptors and DNA damaging anti-cancer regimens
Chapter 6

Summarizing Discussion
SUMMARIZING DISCUSSION

It was recently appreciated that conventional anti-cancer regimens, like γ radiation and chemotherapeutic drugs, can kill cells by activating apoptotic signaling pathways. Since apoptosis resistance is one of the 'hallmarks of cancer', it is thought that tumor cells can become resistant to anti-cancer therapies by gain of apoptosis inhibitors or loss of apoptosis inducers. Insight in the molecular mechanisms of the utilized apoptosis pathways can help to design novel types of therapy that selectively induce apoptosis in tumor cells and protect normal healthy cells. The research presented in this thesis was aimed to define key mediators involved in communication to the mitochondria in apoptosis signaling induced by death receptors as well as DNA damaging anti-cancer regimens. The leukemic T cell line Jurkat was chosen as the main model system, since these cells display sensitivity to death receptor stimulation as well as to DNA damaging anti-cancer regimens, including γ radiation and the chemotherapeutic drug etoposide. Our lab generated CD95-resistant variant clones from a wild type Jurkat clone that proved cross-resistant to apoptosis induction by etoposide and γ radiation failed to release Cyt c from the mitochondria. This indicates the presence of common aspects in apoptosis signaling by CD95, etoposide and γ radiation. Thus, we pinpointed at which level the mitochondrial activation was affected and what the common and distinct aspects are in these signaling pathways.

CD95 versus TRAIL-receptor apoptosis signaling to the mitochondria

Recently death receptors were explored in more detail as anti-cancer tools, since their apoptosis pathways can evade certain inhibitions at the mitochondrial level that are frequently found in tumor cells (see also Figure 1 in chapter 1). For the CD95 signaling pathway it has been established that FADD, Caspase-8 and −10 are recruited to the DISC formed at the receptor tail\(^1\). Subsequent activation of these inducer caspases could lead to direct activation of effector caspases or to cleavage of the Bcl-2 BH3-only family member Bid, which signals to the mitochondria to activate the extrinsic pathway. TRAIL receptor signaling pathways on the other hand were far from elucidated. FADD-deficient cells demonstrated that FADD is required for apoptosis induction by both TRAIL-R1 and -R2\(^2\). Also Caspase-8 and −10 have been found in native TRAIL receptor complexes and Caspase-8 requirement was shown in Caspase-8 deficient cells\(^3\sim5\). TRAIL receptors can also signal to mitochondria as revealed by Cyt c release and mitochondrial depolarization, but the exact sequence of events had not been established.

The apoptotic execution in response to death receptor stimulation in the Jurkat cells used in this study is dependent on the mitochondrial amplification loop for effector
caspase activation, since Bcl-2 overexpression severely reduced the apoptotic response. This made these cells ideal to investigate the exact sequence of events in the signaling pathway to the mitochondria in response to TRAIL. The interesting observation that TRAIL receptor still induced Cyt c release in the CD95-resistant variant clones, suggested that CD95 and TRAIL receptors signal differently to the mitochondria (chapter 2). Others have also observed differential sensitivity of various cell types to CD95- and TRAIL-induced apoptosis in different cell lines. To explore the point of divergence of the signaling pathways, we analyzed cells that overexpressed either dominant negative FADD, c-FLIP\textsubscript{L} or a non-cleavable Bid mutant (Caspase-8 and Granzyme B cleavage sites mutated) with respect to apoptosis incidence and Cyt c release in response to both death receptor ligands. Both stimuli failed to induce apoptosis or Cyt c release in these situations. Furthermore, depletion of Bid from TRAIL- or CD95-activated cytosols impeded their capacity to mediate Cyt c release from mitochondria \textit{in vitro}. Thus, we have established that TRAIL receptor, like CD95, requires FADD, caspase-8/-10 and processed Bid to signal to the mitochondria (chapter 2). Apparently, other protein(s) downstream of Bid are involved in the TRAIL signaling pathway because the CD95-resistant variant cells are still capable to release Cyt c in response to TRAIL. Inhibition of RNA and protein synthesis restored CD95 signaling to the mitochondria in the variant cells, suggesting that the communication to the mitochondria might be inhibited by a protein of high turn over and argues against a loss of function mutation in these cells.

We used an \textit{in vitro} assay to examine whether lack of Cyt c release in CD95-resistant cells was due to an impediment in the cytosol or intrinsic to mitochondria (chapter 2). Addition of recombinant Caspase-8 allowed the generation of truncated Bid (tBid) from full length endogenous Bid in cytosols prepared from resting wild type Jurkat cells and gave efficient release of Cyt c from exogenous mouse liver mitochondria. However, recombinant Caspase-8 was not able to release Cyt c in the presence of cytosol from CD95-resistant cells. Nevertheless, a similar amount of endogenous tBid was generated in this cytosol and tBid targeted the mitochondria as efficiently as in the wild type situation. Also, recombinant tBid lacked the ability to release Cyt c from purified mitochondria in the presence of cytosol prepared from resistant cells. Thus, the apoptosis signaling pathway leading to Cyt c release is blocked by a cytosolic protein that either blocks tBid function after its insertion into the mitochondria or interferes with the function of another factor which collaborates with tBid to bring about Cyt c release. TRAIL receptor signaling can bypass or neutralize the activity of this inhibitory cytosolic factor that blocks tBid function, which might be a powerful mechanism to break apoptosis resistance in tumor cells, since the apoptotic response to DNA damaging anti-cancer regimens is also abrogated in these CD95-resistant variant cells.
Summarizing Discussion

Cytosolic Bax is a common accessory in both TRAIL receptor and CD95 signaling to the mitochondria, as depletion of Bax from cytosol of stimulated cells reduced but not fully abrogated the release of Cyt c from purified mitochondria (chapter 2). Although the effect of Bax depletion in TRAIL activated cytosols is stronger than in CD95 activated cytosols, prolonged incubation with mitochondria clearly showed release of Cyt c. Immunoblotting on lysates of CD95-resistant variant clones revealed similar Bak protein expression levels as compared to wild type Jurkat cells, however, no or dramatically less Bax was expressed in some resistant clones (unpublished data). Yet, these cells undergo TRAIL-induced Cyt c release in vivo. This is consistent with the reported redundancy between Bax and Bak\textsuperscript{6,8}. There is some discrepancy in literature about this point, since recent data showed that Bax deficient human HCT116 colon cancer cells do not undergo TRAIL-induced apoptosis, whereas Bak deficient cells do\textsuperscript{9}. Another study stressed that at least one functional Bax allele is required for TRAIL-induced apoptosis in colon cancer cells, but it should be mentioned that these cells were deficient for Bak\textsuperscript{10}.

Recently, new modulators were identified via RNA interference-based phenotypic screening in TRAIL-induced apoptosis in HeLa cells\textsuperscript{11}. One novel gene (FLJ32312; renamed DOBI) is of particular interest since the data suggested that DOBI acts downstream of tBid but upstream of Cyt c release. It is of interest to examine whether this DOBI protein is restricted to TRAIL signaling or also implicated in CD95 signaling or even in other apoptosis pathways. Future experiments on its mechanism of action and its localization in cells will provide interesting information to better understand how death signals are conveyed to mitochondria. Moreover, new screens with a bigger library of RNA interference constructs, such as the one made at the Netherlands Cancer Institute, should reveal novel genes and/or provide more insight in TRAIL-receptor apoptosis signaling.

DNA damage-induced apoptosis signaling to the mitochondria

Previously, it has been proposed that for some cell types anti-cancer drugs can increase expression of death receptors and/or ligands and thereby induce apoptosis via the death receptor pathways\textsuperscript{12-14}. However, the relative importance for such a mechanism to induce apoptosis in general is doubted, since for instance etoposide\textsuperscript{15} and \(\gamma\) radiation-induced\textsuperscript{16} apoptosis is independent of CD95 in murine thymocytes and Jurkat cells\textsuperscript{17}. In chapter 4 we confirmed by retroviral transduction with dominant negative FADD and c-Flip\textsubscript{L} that DNA damaging anti-cancer regimens induce apoptosis in Jurkat T cells in a death receptor-independent manner. A PCR-based assay that allows quantitative comparison of multiple transcripts of known apoptosis mediators amplified in one reaction (MLPA) was used to analyze transcription in response to DNA damage. Puma was the only gene in this set, that included all known Bcl-2 family proteins, that was
modestly upregulated compared to undetectable levels in unstimulated cells. However, Puma protein was not reliably detectable, RNA interference had no effect on the sensitivity towards DNA damaging agents and depletion of Puma from activated cytosols in in vitro experiments had no influence on their ability to release Cyt c from purified mitochondria (chapter 4 and unpublished data). Thus, we did not obtain an indication that Puma or other known apoptosis genes, including the Bcl-2 family, were transcriptionally upregulated (or downregulated) in response to DNA damage in our cells. Another possibility to identify (novel) genes that are involved in the DNA damage pathway would be to compare RNA from unstimulated cells with stimulated cells in a micro-array setting, such as the one available at the Netherlands Cancer Institute.

As mentioned earlier, CD95-resistant variant clones are also resistant to etoposide and γ radiation-induced apoptosis and have an inhibition of tBid function. Only a few hints in literature indicate that Bid might play a role in DNA damage responses. It was found that Bid−/− mice are prone to develop malignancy resembling chronic myelomonocytic leukemia, indicating that Bid might act as a tumor suppressor\textsuperscript{18}. There is also indication that Bid is a sensor of DNA damage in some tissues since Bid mRNA was induced in a p53-dependent manner\textsuperscript{19}. However, the novel finding that Bid is unambiguously functional in anti-cancer drug- and γ radiation-induced apoptosis is what we have shown in chapter 4. In vitro Bid depletion experiments and overexpression of non-cleavable Bid, which acts as a dominant negative Bid protein, indicated that aspartate-cleaved Bid is required for DNA damage-induced apoptosis signaling to the mitochondria. We ruled out the known mammalian aspartate-specific proteases (Caspase-8, -3, -2 and Granzyme B) as the one responsible for cleaving Bid in the DNA damage pathway. Recently Caspase-2 was suggested as a key candidate that controls signaling to the mitochondria in response to DNA damage in several cell types\textsuperscript{30}. However by making use of a similar Caspase-2 RNA interference approach as described in literature, we could not demonstrate an essential contribution for Caspase-2 upstream of the mitochondria in our Jurkat cells (chapter 4). Since the non-cleavable Bid mutant appears to act as a dominant negative protein, it may bind with high affinity to the upstream protease involved in its cleavage. Pull-down experiments or immunoprecipitations using the non-cleavable Bid mutant may allow the identification of the protease involved in the DNA damage pathway in Jurkat cells.

Inhibitory protein in CD95, etoposide and γ radiation signaling to mitochondria

The fact that tBid, besides its role in death receptor signaling, also plays an important role in the DNA damage pathway towards the mitochondria and that tBid function is inhibited in the CD95-resistant clones explained why these cells are cross-resistant to DNA damaging anti-cancer regimens. We searched for Bid interacting proteins to elucidate which inhibitory protein was responsible for the block on tBid function in the
resistant cells. Multiple Bid interacting proteins were found in anti-Bid immunoprecipitates or GST-Bid fusion protein fishes. However, these did not differ in expression between wild type and resistant cells, also not after apoptotic stimulation (unpublished data). In addition, attempts to remove the inhibitory action from resistant cytosols by GST-Bid fusion protein fishes failed because GST on its own activated cytosol in general (unpublished data). Yeast-two-hybrid screens with full length Bid as bait repeatedly yielded Bfl-1 as an interacting protein (discussed below), but no other proteins.

RNA and protein synthesis inhibition restores the sensitivity in the resistant cells, indicating that the inhibitory protein is subject to high turnover and might be overexpressed in resistant cells. A PCR-coupled subtractive hybridization was used to identify the overexpressed inhibitory protein in resistant cells, but this had insufficient resolving power. Furthermore, we used a micro-array experiment (the Lymphochip, NCI, US) to look for differential protein expression, but this failed to shed light on the possible inhibitory protein. However, a further attempt with the micro-array resources from the Netherlands Cancer Institute might still be worthwhile. Also the MLPA with the apoptosis gene set did not reveal major differences between wild type and resistant cells (unpublished data).

Recently, Renshaw and colleagues identified novel isoforms of Bid that are all expressed in Jurkat cells\(^1\). \(\text{Bid}_5\), which contains the N-terminal regulatory domains of Bid without the BH3 domain, abrogated the pro-apoptotic effects of tBid and inhibited CD95-induced apoptosis in HepG2 cells. Whether \(\text{Bid}_5\) could account for the inhibitory effect in the resistant cells is unclear, since it is not known what the effect of \(\text{Bid}_5\) is on TRAIL- or DNA damage-induced apoptosis, as its mechanism of action is unknown. A direct binding with Bcl-2 or Bax in functional assays could not be established, which is in line with the lack of the BH3 domain in \(\text{Bid}_5\).

In theory, it cannot be ruled out that the resistance is achieved by a mutation in a protein of high turnover to gain an inhibitory function. We examined PKB/Akt in particular since it can interfere at different steps in apoptotic pathways\(^2\), however constitutive active overexpression of myristoylated PKB was only able to inhibit etoposide-induced apoptosis, thus it did not fit the criteria of the inhibitory factor which in addition also blocks CD95- and \(\gamma\) radiation-induced apoptosis (unpublished data).

To identify apoptosis inhibitors/mediators in a more general setting, screens with retroviral libraries with apoptosis or Cyt c release as read out or screens with a RNA interference library can be carried out. We have set up conditions for efficient expression of retroviral constructs by introducing the ecotropic receptor in wild type Jurkat cells. Some generated clones revealed nearly 100% infection efficiency, as tested by infection with GFP-expressing retrovirus. Preliminary work indicates that the spontaneous resistance to recombinant TRAIL is extremely low (less than 1 in 3 million) in these cells, whereas almost 1 in 1 hundred cells are resistant for CD95 as
Chapter 6

tested for different tested clones and conditions (unpublished data). RNA interference technology in particular is a powerful tool to identify mediators involved in the investigated apoptosis pathway, since it is not dependent on full length expression or expression of the essential domains of genes, taking into account that at least one of the few designed interference sequences directed against a given gene is functional.

Bcl-2 family members and their mechanism of action

BH3-only proteins convey the death signal to mitochondria and induce loss of mitochondrial membrane integrity in collaboration with the pro-apoptotic Bax and Bak proteins. Anti-apoptotic Bcl-2 members can prevent this. In an attempt to obtain more insight in the regulation of tBid function, whose function is inhibited in the CD95-resistant variant Jurkat clones, we did a screen for Bid binding proteins. With it, we identified the anti-apoptotic Bcl-2 family member Bfl-1/A1 as a Bid interacting protein. Bfl-1 is able to block apoptosis and Cyt c release in response to DNA damage and CD95 stimulation, but also in response to TRAIL receptor stimulation (chapter 3). Therefore, Bfl-1 is not the cytosolic factor responsible for the resistance observed in the CD95-resistant variant clones, since these are still sensitive to TRAIL. However, we obtained more insight in the mechanism of action of the apoptosis inhibitory Bfl-1 protein. The fact that Bfl-1 did not inhibit Bid processing nor tBid’s association with the mitochondria indicated that it either directly inhibits tBid function or inhibits this indirectly by inhibiting another factor that is required for Cyt c release (regardless of its interaction with tBid).

As demonstrated in chapter 3, Bfl-1 preferentially binds to tBid and not to pro-apoptotic Bax or Bak in the plane of the mitochondrial membrane, thereby it sequestered tBid and abrogated the synergism between tBid and Bax or Bak. In addition, trace amounts of tBid and Bax or Bak complexes were found, suggesting that they interact but that this might be of a transient nature. Thus, our data fit with the sequestration models A and/or C presented in chapter 1 in Figure 2. So far, for death receptor-induced apoptosis signaling only tBid is implicated in signaling to the mitochondria, therefore it is difficult to distinguish whether model A or model C is the correct one, since tBid is considered as an activator BH3-only protein and thus can activate pro-apoptotic Bax and Bak without the help of certain sensitizer BH3-only proteins. Addition of different recombinant BH3-only proteins, including tBid, BimL, Bmf, Puma, Noxa and Bad, to purified mitochondria in vitro indicated that all investigated BH3-only proteins were able to induce release of Cyt c from these mitochondria, however tBid was the most potent inducer followed by BimL and Puma, which supports to some extent model C as described in chapter 1 (unpublished data).

The fact that both Bfl-1 and Bcl-2 can protect from CD95-, TRAIL-R1/2- and as previously described TNF-R1-induced apoptosis^{23,25} can be explained by their
Summarizing Discussion

capacity to inhibit the apoptotic activity of tBid. Although Bfl-1 and Bcl-2 were equally efficient in preventing apoptosis induction by death receptor stimulation, Bfl-1 was less efficient in blocking DNA-damage-induced apoptosis than Bcl-2 in Jurkat cells (chapter 3). This raises the question whether anti-apoptotic Bcl-2 type members display differential binding specificities to the different BH3-only proteins. As shown in chapter 4, tBid plays a very crucial role upstream of the mitochondria in DNA damage-induced apoptosis as well. Still, Bcl-2 is more potent than Bfl-1 in inhibiting DNA damage-induced apoptosis. The intriguing thing is that in vitro binding studies from solubilized mitochondria to which recombinant proteins were added, indicated that Bfl-1 binds much stronger to tBid than Bcl-2 does (unpublished data). This suggests that other BH3-only proteins, besides tBid, also might play a role in the response to DNA damage which can be more efficiently inhibited by Bcl-2, although blocking of tBid production by either overexpression of a non-cleavable Bid mutant or depletion of Bid from activated cytosols abrogated the capacity of the cells to release Cyt c in response to DNA damage. We did observe that Puma binds more efficiently to Bcl-2 than to Bfl-1 (unpublished data), and that Puma is upregulated on RNA level in response to γ radiation and etoposide in our Jurkat cells, however on protein level it was not reliably detectable and RNA interference of Puma, with known inhibitory capacity in other cells, did not have any effect. As a consequence, it is unlikely that Puma is another possible BH3-only protein that functions alongside to tBid for DNA damage-mediated apoptosis in the Jurkat cells, in contrast with its essential role in thymocytes\(^{26,27}\). Also gene knock out in human colorectal cancer cells showed that Puma was required for apoptosis induced by p53, hypoxia and DNA damaging agents\(^{28}\). There are some studies that suggest that a differential binding capacity between BH3-only proteins and anti-apoptotic Bcl-2 members exist as tBid, Bim\(_L\) and Bad appeared to interact differently with Bcl-2 and Bcl-x\(_L\)\(^{26-32}\).

Modulators of apoptosis as target for anti-cancer therapy

Most anti-cancer agents induce apoptosis, thus defects in apoptotic programs may contribute to treatment failure\(^{33}\). Therefore, cancer genetics are linked with cancer therapy. As impaired apoptosis is suggested to be one of the hallmarks of cancer, the search for strategies to enhance apoptotic cell death is intense. It is favored that these treatments directly induce apoptosis, since this enhances the effective outcomes, reduces the chance of toxicity, and decreases mutagenesis. As a logical consequence, these strategies aim to limit the function of oncogenes such as Bcl-2 and IAPs, or to enhance the activity of tumor suppressors like Bax and BH3-only proteins, or to engage specific apoptotic pathways like the death receptor pathway\(^{34}\). Thus, the goal of anti-cancer therapy should cover effectivity, selectivity and the capability to bypass resistance in tumor cells.

107
Since overexpression of Bcl-2 and other anti-apoptotic family members in a number of tumor mouse models contribute to oncogenesis and inhibition of apoptosis is seen in many cultured cells upon treatment with chemotherapeutic drugs (chapters 3, 4), and since Bcl-2 type proteins are known to function downstream of the frequently mutated tumor suppressor protein p53, it makes it tempting to directly inhibit their function. Clinical studies with intravenous infusion of antisense oligonucleotides targeting Bcl-2 or Bcl-xL in combination with other anticancer treatments, including chemotherapy and γ radiation, yield promising results by e.g. reverting chemoresistance. Because the results obtained with antisense oligonucleotides are not always that specific and reliable, perhaps the use of the RNA interference would be more effective when efficient and safe delivery is possible. Another approach to directly target more than just one (or just one) Bcl-2-type family member and reduce their function is the introduction of BH3 mimetics. These small compounds mimic the interaction of a BH3 domain with its target groove in Bcl-2-type proteins. However, to date only in vitro preclinical data is obtained using hydrophobic carboxy-terminal truncated prosurvival Bcl-2 family members or small-molecule cell-permeable chemicals. Thus, more research is required to judge their effectiveness in vivo before these BH3 mimetics will be clinically useful.

To note, any compound or molecule that targets any of the pro-survival or pro-apoptotic BH3-only Bcl-2 family proteins will only work if at least either Bax or Bak is present, since cells lacking both Bax and Bak are dramatically impaired in apoptosis and are resistant to BH3-only induced cell death. Furthermore, delivery of molecules should somehow be restricted as much as possible to tumor cells and let normal healthy cells untouched. Even though the upcoming research for replication-selective oncolytic adenoviruses as new tools for anti-cancer therapy, like Onyx-015, is growing, the delivery of genes by adenoviruses will probably not be efficient enough. Thus, the chances of gene therapy in general as successful new anti-cancer therapies are low.

Death receptor-induced apoptosis pathways can directly activate effector caspases, thus they can bypass certain inhibitions at the mitochondrial level that frequently occur in tumor cells and are not affected by loss of functional alleles of p53. Death ligands can work synergistically with DNA damaging anti-cancer agents and can overcome certain drug resistance in tumor cell lines because both the extrinsic and intrinsic apoptosis pathways are activated. Although TNFα and CD95L are potent death ligands that induce apoptosis in many cell lines, they are precluded for systemic therapy in vivo due to their severe toxic effects: lethal inflammatory responses and liver cell death, respectively. Recombinant TRAIL on the other hand shows great potential as anti-cancer therapeutic agent, since it is capable of killing many human tumor cell lines whilst sparing normal cells, which are resistant to TRAIL. Furthermore, systemic administration to animals in mouse and monkey preclinical models showed no toxicity. In addition, p53 is a transactivator of TRAIL-R2, thus in case of functional
p53. DNA damaging drugs upregulate its receptor, which might enhance the efficacy of the treatment when TRAIL is combined with conventional anticancer therapies. Therefore, systemic administration of TRAIL has the power to reach in theory every cell in the organism and is selective in its killing for tumor cells as well, making it a potential candidate for anti-cancer treatment.

Although far from preclinical study-level yet, the capacity of Drosophila Reaper to induce apoptosis by general suppression of protein translation in human cells might be an interesting tool as well. In chapter 5 we described that Reaper can activate caspases and induce apoptosis in human cells independently of mitochondrial permeabilization, Cyt c release and of its IAP binding motif. Deletion of its twenty amino acids from the carboxy-terminus fully abrogated its potential to inhibit protein synthesis and to induce apoptosis. Thus, suggesting that the capacity of Reaper to suppress protein translation can operate in mammalian cells and may be key to its pro-apoptotic activity. Further experiments are required to establish whether apoptosis-induction in vivo and translational inhibition are separable or structurally linked, since expression of just the carboxy-terminal twenty amino acids of Reaper failed to suppress protein translation, but also failed to induce apoptosis. If the exact mechanism of translational inhibition of Reaper is elucidated in the future, perhaps small-molecule cell-permeable compounds can be designed as anti-cancer tools that mimic this mechanism. Inhibition of protein synthesis is involved in germline apoptosis of C. elegans as well, suggesting an evolutionary relevance for such a mechanism (M. Hengartner, personal communication). Also Reaper's ability to suppress the anti-apoptotic function of IAPs might be interesting. Recently, Schimmer and colleagues showed that a small-molecule compound that specifically antagonized XIAP's inhibition of effector caspase-3 directly induced apoptosis in many types of tumor cell lines and sensitized cancer cells to chemotherapeutic drugs. Also growth of established tumors was suppressed in xenograft models in mice, while displaying little toxicity to normal tissues.58

As described above, various novel anti-cancer therapies can be designed to directly induce or enhance apoptosis selectively in tumor cells and by that reduce toxicity to normal tissues.

**Concluding remarks**

This thesis provides new insights in key mediators involved in communication to mitochondria in apoptosis signaling induced by death receptors as well as DNA damaging anti-cancer regimens. TRAIL receptor signaling, like CD95, requires FADD, DED-caspases and cleaved Bid. Interestingly, presynthesized aspartate-cleaved Bid plays also a crucial role in DNA damage apoptosis signaling to mitochondria, but the protease in this pathway is not identified yet. In addition, we have established that a
cytosolic inhibitory factor blocks tBid function in CD95-resistant Jurkat clones, which explains why these clones proved cross-resistant to apoptosis induction by etoposide and γ radiation. TRAIL receptor signaling has the ability to circumvent the block on tBid function in these clones, further stressing its potential as anti-cancer candidate drug. Furthermore, the characterization of the mechanism of action of the anti-apoptotic Bfl-1 protein support the idea that anti-apoptotic Bcl-2 members regulate mitochondrial apoptotic activation by sequestering BH3-only proteins and by that block the collaboration between BH3-only proteins and pro-apoptotic Bax or Bak. Also, the newly identified capacity of Drosophila Reaper to induce apoptosis by general suppression of protein translation in human cells provides interesting information on novel mechanisms to induce apoptosis. Especially in view of the fact that Reaper’s ability to induce apoptosis was independent of the mitochondria and thus not influenced by overexpression of anti-apoptotic Bcl-2 members, which is frequently observed in tumor cells.

Reference List


Summarizing Discussion


16 Smith KG, Strasser A, Vaux DL. CrmA expression in T lymphocytes of transgenic mice inhibits CD95 (Fas/APO-1)-transduced apoptosis, but does not cause lymphadenopathy or autoimmune disease. EMBO J 1996; 15(19):5167-5176.

Chapter 6


31 Terradillos O, Montessuit S, Huang DC, Martinou JC. Direct addition of BimL to mitochondria does not lead to cytochrome c release. FEBS Lett 2002; 522(1-3):29-34.
Summarizing Discussion


Chapter 6


