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COMMENTARY

Apoptosis in the development and treatment of cancer

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Our somatic cells are born by mitosis and almost all will die by apoptosis, a physiological process of cellular suicide. Cancers can occur when this balance is disturbed, either by an increase in cell proliferation or a decrease in cell death. The goal of cancer therapy is to promote the death of cancer cells without causing too much damage to normal cells. Our knowledge of the mechanisms of apoptosis has enhanced our understanding of how some cancers originate and progress. It has also revealed that existing cancer therapies can work in two ways, by induction of apoptosis as well as by direct toxicity. In some cases resistance to apoptosis may explain why cancer therapies fail. Novel treatments designed to exploit our knowledge of apoptotic mechanisms are under development to promote apoptosis of cancer cells and limit concurrent death of normal cells.

Introduction

Apoptotic cells have long been observed in cancers. For example, the high rate of apoptosis seen in basal cell carcinomas of the skin explained why these are relatively slow growing tumors, in spite of their high mitotic rate (1). Increased apoptosis was observed in irradiated tumors and those treated with cytotoxins, implying that treatments that increased the rate of apoptosis could be used to treat cancers (2).

However, the role of failure of apoptosis in causing cancers was only realized much later. This followed the discovery that the *bcl-2* gene, which is often translocated in follicular lymphoma, encoded a cell death inhibitory protein (3). As the first component of the apoptotic mechanism to be identified, *bcl-2* also helped in the elucidation of other parts of the apoptotic mechanism.

When *bcl-2* was expressed in cells in tissue culture, it not only protected them from apoptosis due to removal of growth factors, it also prevented apoptosis following treatment with a diverse range of drugs and toxins, giving cells a multidrug resistance phenotype (4,5). This suggested that apoptosis inhibitory genes such as *bcl-2* might not only play a role in the development of malignancy, but also determine the response to therapy.

On its own, however, inhibition of apoptosis does not rapidly transform cells or cause cancers. However, when inhibition of apoptosis, by Bcl-2 for example, is combined with activation of a conventional growth stimulatory oncogene such as c-*myc*, cancers can develop very rapidly (6).

Mechanisms of apoptosis

Not all cell death is physiological

It is axiomatic that all cells will die if a process necessary for their continued survival is blocked. In addition to being mortal, most animal cells can also be suicidal, meaning that they bear mechanisms whose physiological role is to cause their own death. One such physiological cell suicide process is termed apoptosis or programmed cell death. Cells that kill themselves by implementing this process typically exhibit a characteristic morphology.

Usually, apoptotic cells shrink, their chromatin condenses around the margins of the nucleus and *in vivo* the cell is engulfed by another cell. Biochemical markers of apoptosis include activation of proteases termed caspases, cleavage of proteins and DNA and exposure of phosphatidylserine on the cell surface. Although these events are helpful in identifying cells that have undergone apoptosis, it is important to note that they may occur well after a cell has committed to die and some cells that have activated the cell death program and are destined to die may not exhibit any of these changes (7).

While it is easy to determine if a cell is dead, it is much harder to determine if it is still alive. Although it is not applicable to all cell types, the gold standard for determining if a cell has died is loss of the potential to reproduce to generate a clone (8). Of course, with cancer cells this is of particular importance.

Apoptosis is a common stress response

Further complicating the analysis of cell death is the fact that apoptosis is a common response to cell stress (9). Cells monitor many aspects of their physiology. Any drug or agent that is capable of killing a cell will cause physiological changes when given at sub-lethal doses or in the period before the cell is biochemically inert. When detected by the cell, these changes often elicit some kind of stress response. Some responses, such as production of heat shock proteins, may serve to protect the cell, whereas others, such as activation of the apoptotic process, may hasten its demise. The ability of drugs and toxins with known lethal biochemical activities to nevertheless provoke an apoptotic death response has caused a great deal of confusion in the field.

Not only drugs can induce an apoptotic response, but by disturbing cell physiology, so can loss of gene expression, overexpression of genes and expression of mutant genes. The oncogene c-*myc*, for example, can stimulate apoptosis both when it is overexpressed (10,11) or when its expression is suddenly reduced (12). The regulation of apoptosis is not an intrinsic function of the great majority of drugs and genes, but if the goal of a drug is to cause death of cancer cells, its ability to cause cell suicide indirectly might be just as important as its direct cytotoxic activity.

The key biochemical events in apoptosis

Given that there now exist more than 80 000 publications on apoptosis, it is surprising to have to admit that the key event that causes cell death is still hotly debated. In the nematode *Caenorhabditis elegans* the key event in programmed cell death is activation of a protease termed CED-3 and cleavage of its substrates (13,14). No programmed cell death occurs in worms with mutations to *ced*-3. Mammals have about a dozen homologs of CED-3 that are collectively termed caspases (15,16). While some believe that caspase activation will also turn out to be the key essential event in apoptosis in mammalian cells, the majority of researchers currently believe that in most cases of apoptosis the key event is mitochondrial failure due to loss of cytochrome c from the mitochondrial intermembrane space (17,18).

During programmed cell death in *C.elegans* the protease CED-3 is activated by the adaptor protein CED-4. The mammalian protein caspase 9 most closely resembles CED-3 and caspase 9 can be activated by Apaf-1, an adaptor protein that resembles CED-4, the worm adaptor required for activation of CED-3 (19–21).

In mammalian cells Apaf-1 is activated by cytochrome c after it is released from the mitochondria (22). Apaf-1 proteins then form a multi-subunit complex with caspase 9 termed the 'apoptosome' in which caspase 9 becomes proteolytically active (23). Caspase 9 can cleave and activate other caspases such as caspase 3, which cleaves many proteins within the cell, including ICAD, an inhibitor of an endonuclease (CAD) that cleaves the cell's DNA (24), which can be detected as the classical ladder pattern by electrophoresis (25,26).

Although Apaf-1 resembles *C.elegans* CED-4 and caspase 9 resembles CED-3, there are crucial differences. Unlike Apaf-1, CED-4 does not have to be activated by cytochrome c, and although CED-4 and CED-3 are essential for programmed cell death in the worm, developmental cell death occurs more or less normally when the genes for Apaf-1 or caspase 9 are deleted in mice, especially when they are crossed onto the C57/Bl6 background (27–29).

Experiments in cell lines derived from mice lacking the gene for Apaf-1 or caspase-9 show that although they are needed for rapid exhibition of the apoptotic phenotype, they do not affect clonagenic potential of factor-starved or chemotherapy-treated cells (7). Thus, even though lack of Apaf-1 and caspase-9 delayed appearance of the apoptotic phenotype in cell death caused by retinoblastoma protein or cytotoxic drugs (30), it did not necessarily affect the number of cells that ultimately died.

Unlike the worm, mammals have an additional cell death pathway that is controlled by certain members of the tumor necrosis factor (TNF) receptor family, often referred to as 'death receptors'. In this pathway, when receptors such as TNF receptor 2, CD95 (Fas/APO-1) and TRAIL receptors are ligated, their cytoplasmic domains recruit the adaptor protein FADD, which in turn recruits and activates caspase 8 and caspase 10 (31,32). Although activation of these caspases can cause secondary damage to the mitochondria, in most cells they can cause cell death independently of the mitochondria (33,34).

Apoptosis is regulated by Bcl-2 family members

In *C.elegans* programmed cell death is inhibited by CED-9, which directly binds to CED-4, preventing it from activating CED-3. Mammals have many CED-9 like proteins, known collectively as the Bcl-2 family, but none of these proteins interacts directly with Apaf-1, the mammalian protein most similar to CED-4 (35). While there is general agreement that Bcl-2 family members, including Bcl-2 itself, Mcl-1, Bcl-x and Bcl-w, can inhibit apoptosis, provide clonagenic protection and act upstream of the mitochondria, exactly how they work is not known (36). Gene deletion experiments provide evidence that the apoptosis pathways that can be inhibited by Bcl-2 require either Bax or Bak, two pro-apoptotic Bcl-2 family members, to function (37). *Caenorhabditis elegans* does not have any pro-apoptotic Bcl-2 family members, so provides few clues as to how they function. One model is that when an apoptotic signal is received, Bax and Bak aggregate on the mitochondrial outer membrane and form channels that allow cytochrome c to escape (38).

In addition to the anti-apoptotic Bcl-2 family members and the pro-apoptotic family members Bax and Bak, there is a third subfamily of Bcl-2-like proteins known as 'BH3-only' proteins because they bear only one of the Bcl-2 homology domains, BH3 (39). These are pro-apoptotic proteins. In *C.elegans* the BH3-only protein EGL-1 promotes cell death by binding to CED-9 and causing it to release CED-4, which can then activate the caspase CED-3 (40). In mammalian cells BH3-only proteins such as Bim, PUMA, Bid and Bad (and others) can bind to anti-apoptotic Bcl-2 family members such as Bcl-2, Mcl-1, Bcl-w and Bcl-x, but how this leads to activation of Bax and Bak remains mysterious.

The BH3-only proteins are controlled by transcription, phosphorylation, sequestration and cleavage. For example, the tumor suppressor gene *p53* induces apoptosis by transcriptionally activating the gene for PUMA, which can bind to and antagonize anti-apoptotic Bcl-2 family members (41,42). Bmf and Bim are kept inactive in healthy cells by sequestration on microtubules and myosin, respectively (43,44). Bid is activated following cleavage by caspase 8 (45) and Bad is regulated by phosphorylation (46).

Inhibitor of apoptosis proteins (IAPs) can regulate cell death after caspase activation

In addition to proteins such as Bcl-2 that inhibit cell death upstream of the mitochondria and prior to activation of the caspases, there is another family of proteins termed IAPs that act after caspases become activated by binding to them and preventing them from cleaving their substrates (47). All IAPs bear one or more baculoviral IAP repeat (BIR) domains and most also have a RING domain that allows them to act as E3 ubiquitin ligases. The most well-characterized mammalian IAP is XIAP, which can bind to and inhibit caspases 3, 7 and 9 via its BIR domains (48).

Analysis of an IAP in *Drosophila*, DIAP1, has shown that it is antagonized by the small pro-apoptotic proteins Reaper, Grim, HID and Sickle (49,50). These proteins bind the BIR domains of DIAP via an interaction motif found in their N-termini. Mammals also have a number of IAP-binding proteins that have similar N-termini, but unlike the *Drosophila* IAP antagonists, which are cytoplasmic, most of the mammalian ones reside in the mitochondria in healthy cells (51). Experiments using recombinant proteins have shown that inhibition of caspases by IAPs can be relieved by addition of these IAP antagonists *in vitro*.

Inhibition of apoptosis can lead to cancer

The evidence that inhibition of cell death can lead to cancer comes mainly through accidents of nature, such as

translocations in lymphomas and leukemias. For some cell death genes knockout and transgenic mice have provided evidence confirming that failure of cell death can cause cancer. Although there have been a very large number of studies determining the levels of expression of genes for cell death inhibitors in various types of cancer, these studies only provide correlative evidence.

Follicular lymphoma and Bcl-2

The most common cancer of the blood cells in humans is the B cell neoplasia follicular lymphoma. The *bcl-2* gene was identified because it lies at the breakpoint of the t(14;18) translocation that is found in most cases. Experiments *in vitro* showed that Bcl-2 can prevent apoptosis of cells starved of cytokine and revealed it to be the first oncogene that acts by inhibiting cell death rather than by stimulating cell proliferation (3).

Expression of Bcl-2 in transgenic mice confirmed that inhibition of apoptosis can lead to cancer, as these mice develop B cell lymphomas and leukemias (6,52–54). However, although Bcl-2 is a very potent inhibitor of apoptosis, *bcl-2* transgenic mice only develop cancers when they are very old. These results suggest that inhibition of cell death is only very weakly oncogenic or, put the other way, apoptosis of potential cancer-forming cells is not a potent suppressor of tumorigenesis, at least in mice.

Although on its own expression of a *bcl-2* transgene in the lymphoid compartment of transgenic mice did not lead to the rapid development of tumors, when combined with a c-*myc* transgene leukemias developed extremely rapidly, much more rapidly than in mice bearing either transgene alone (6). This potent synergy between a growth-inducing oncogene and a cell death inhibitor may indicate that apoptosis is important in preventing survival of cells that already have activating mutations in growth-promoting oncogenes. These results imply that cells can detect disturbances caused by an activated oncogene and engage the apoptotic mechanism. Blocking apoptosis by overexpression of Bcl-2 or loss of a component of the signal transduction pathway (such as p53) that connects the damage sensors to the cell death mechanism would greatly facilitate development of malignancy.

p53

p53 is the most commonly mutated gene in human cancers, but precisely how it prevents tumors developing is not certain. Humans heterozygous for loss of function mutations to *p53* (Li–Fraumeni syndrome) and mice with one or both alleles of *p53* deleted develop cancers at an early age in many different tissues (55,56). p53 has two main functions, it can cause cell cycle arrest by transcriptionally activating the *p21* cyclin kinase inhibitor gene (57) and it can cause apoptosis by transcriptionally activating pro-apoptotic genes, especially for the BH3-only protein PUMA (41,58).

Whether induction of apoptosis or cell cycle arrest is important for the tumor suppressor activity of p53 is unclear. Although lymphoid cells in *bcl-2* transgenic mice are just as resistant to apoptosis due to ionizing radiation and DNA mutagens as those from *p53* mutant mice (59), the *bcl-2* transgenics have a far lower incidence of lymphoma (52). Similarly, mice lacking genes for PUMA or both Bax and Bak do not develop cancer with anything approaching the frequency seen in *p53* null mice, even though p53 requires PUMA to induce apoptosis in response to DNA damage and all p53-mediated apoptosis is thought to require Bax and or Bak.

This implies that the ability of p53 to induce apoptosis is relatively unimportant for its tumor suppressor activity. On the other hand, the incidence of tumors is also not markedly elevated in *p21* deleted mice (60), so this activity of p53 also seems to be relatively unimportant. These findings suggest that there may be an additional activity of p53 that accounts for its ability to act as a tumor suppressor.

Recently it has been suggested that p53 may also act independently of transcriptional regulation to directly bind to and inhibit Bcl-x at the mitochondria, leading to apoptosis (61). Because the gene for PUMA must be induced by p53 following DNA damage and PUMA is required for p53-mediated apoptosis (42), this alternative activity of p53 seems unlikely to represent a major aspect of the pro-apoptotic activity of p53 and, therefore, would not be important for p53-mediated tumor suppression.

Experiments studying established tumors expressing c-myc or studying the transformation of cells from mice engineered to overexpress myc give support to the idea that the ability of p53 to cause apoptosis may be important to prevent progression of a tumor after the initial oncogenic event (62). Thus, non-apoptotic activities of p53, such as causing cell cycle arrest, might be necessary to prevent the first oncogenic mutation, whereas its ability to induce apoptosis might allow it to retard subsequent transforming events.

IAPs and paracaspases

A translocation involving genes for cIAP2 and paracaspase commonly occurs in mucosa-associated lymphoid tissue (MALT) lymphomas (63–65). This translocation results in production of a fusion protein with an N-terminal half containing the BIR domains of cIAP2 and the C-terminal half containing the protease-like parts of paracaspase (66). How this fusion protein might contribute to oncogenesis has not been determined, but the possibilities include inhibition of apoptosis by the BIR domains or through activation of NFκB p52 by the paracaspase region. Translocations resulting in increased expression of Bcl10 are also associated with MALT lymphoma (67,68). Because Bcl10 acts directly upstream of paracaspase in a pathway required for activation of NFκB in response to antigen receptor ligation, it seems likely that MALT lymphomas are caused by increased NFκB signaling rather than direct inhibition of caspases by IAPs.

Correlative evidence

For almost all of the pro- and anti-apoptotic proteins there have been studies demonstrating correlations between their expression and various types of cancer. For example, in addition to the follicular lymphomas that bear *bcl-2* translocations, elevated Bcl-2 expression has been associated with progression in melanoma (69) and both it and Mcl-1 have been observed in some myelomas (70,71). Elevated levels of XIAP have been observed in small cell carcinoma of the lung (72,73) and ML-IAP expression seems to be restricted to melanoma cells (74–76).

The problem with these observations is that detection of a protein in cancer cells does not prove that it was involved in causing the cancer or is required for its persistence. The protein Survivin, which was wrongly assumed to be a cell death inhibitor because it bears a BIR domain, is required for chromosome segregation during cell division (77,78). That Survivin can be detected in many cancers but few normal tissues may simply reflect the fact that some cells in all cancers are

dividing, whereas most cells in normal adult tissues rest in G_0 (79). Nevertheless, because cells cannot divide without Survivin, it might turn out to be an excellent target for novel cancer chemotherapeutics: inhibition of Survivin (or its partners INCENP and aurora B) might be as good as or perhaps better than Taxol (78,80).

Evidence from KOs and transgenics: in vivo models/ validation

One can have much more confidence that an inhibitor of apoptosis can act as an oncogene or that a promoter of cell death can act as a tumor suppressor based on findings obtained from transgenic and knockout mouse studies. If transgenic mice made to overexpress an apoptosis inhibitor have an increased incidence of cancer, the oncogenic potential of such a gene can be verified. Increased cancer incidence in mice with a targeted deletion of a gene suspected to be a promoter of cell death confirms the ability of such a gene to act as a tumor suppressor.

Even if gene-deleted mice or transgenic mice do not spontaneously develop cancer, these mice can be crossed onto mice with a sensitized genetic background that are known to develop cancer at a certain rate and the crossed mice can be monitored to see if cancer onset is hastened. For example, mice lacking the gene for the BH3-only protein Bim do not have a high incidence of cancer, but deletion of Bim accelerates the onset of lymphoma in Eμ-myc transgenic mice, arguing that Bim can suppress tumors induced by c-myc (81). Similarly, because deletion of Bax accelerates tumor onset in p53-deficient or c-*myc* transgenic mice, we can be confident that Bax is able to function as a tumor suppressor (82,83). Although studies of the ability of the BH3-only protein PUMA to suppress neoplasia is yet to be demonstrated *in vivo*, experiments *in vitro* suggest it is very likely to be able to do so (84).

The other anti-apoptotic Bcl-2 family members Mcl-1 and Bcl-xl act alone or in combination with other oncogenes to promote cancer when expressed in lymphoid cells (83,85) or pancreatic β cells (12).

Although in this way Bcl-2 family members have been shown to be capable of either promoting or protecting against the development of cancer, so far there has been little *in vivo* validation that other components of the cell death mechanism affect cancer. To date there have been no reports of spontaneous tumor development or even hastening of tumor onset in tumor-prone mice for transgenics expressing other cell death inhibitors or mice in which pro-apoptotic genes are deleted. Mice in which Apaf-1 (86) or caspases 1, 2, 3, 6, 7, 8, 9, 11 or 12 have been deleted do not appear to have increased susceptibility to cancer. Neither do mice transgenic for XIAP or the caspase 8 inhibitor CrmA. That transgenic mice expressing Survivin in their keratinocytes do not spontaneously develop skin tumors or have increased tumor incidence when exposed to carcinogens confirms that *survivin* is not an oncogene (87).

Anticancer agents can induce apoptosis, but most can also kill cells directly

Apoptosis is a common stress response

One of the few areas in the cell death field that everyone does agree upon is that having cancer cells undergo apoptosis would be a good thing. However, there remains debate about how feasible it is to try to get a cancer cell to kill itself by programmed cell death versus trying to kill it directly using a cytotoxic drug (8).

All cells are mortal and all cells can be killed if a process that is necessary for their survival is blocked. Clinically useful chemotherapeutic drugs inhibit processes essential for growth or proliferation, such as blocking production of DNA, mRNA or protein, directly damaging DNA or inhibiting components required for DNA replication or chromosome separation. Cells treated in this way may undergo mitotic catastrophe, lose the ability to maintain plasma membrane activity or become senescent, even if their apoptotic stress response has been incapacitated (88,89).

The same drugs would cause apoptosis in those cells in which the apoptotic mechanism remained intact, leading to cell suicide. A complicating factor in the analysis of cell death is the fact that apoptosis is a common stress response, so that often a cell treated with a potentially lethal toxin will not die because of the direct effects of the toxin, but will activate its suicide mechanism to die earlier by apoptosis (90).

Direct toxicity versus induction of apoptotic stress response

Currently it is unclear how important apoptosis is compared with direct cytotoxicity in the response of cancers to chemotherapy. Because it is not possible to generate a viable mouse lacking all caspases, since mice lacking genes for caspase 8 are inviable (28), it is not possible to study the direct toxic effects of chemotherapy in the absence of an apoptotic stress response. Experiments have been carried out in cells engineered to overexpress Bcl-2 provide part of the answer (4,91). In short-term assays these cells show resistance to many different drugs and radiation, but if higher doses of drug are given or the cells are exposed for long enough, they will still die, exhibiting a necrotic appearance rather than the classical apoptotic phenotype. Far too seldomly, clonagenic experiments are carried out. These are much more important than short-term assays, because they answer the question of whether, at a particular dose of drug, inhibiting apoptosis affects the number of clonagenic cells that survive (8). It is the number of clonagenic cells that will determine whether a tumor can grow.

Therapeutic index and apoptosis of normal cells

Cancers originate from gene mutations in a single cell, but as the progeny of this cell proliferate they are subject to further genetic alterations. The population of cancer cells has to compete for space, nutrients and oxygen, and those that are most robust survive in a process of Darwinian selection. In general, cells that have lost important components of the mechanism for cell death or overexpress cell death inhibitors would have an advantage. Cancer cells that are genetically resistant to apoptosis might also be selected during radiotherapy or chemotherapy. Unlike cancer cells, normal cells have intact programmed cell death mechanisms and, because most treatments designed to kill cancer cells also reach normal cells, they might also undergo apoptosis leading to side-effects to organs, including the gastrointestinal tract, bone marrow and skin.

For these reasons, a cancer therapy that acts solely by the induction of apoptosis and had no intrinsic cytotoxicity would be likely to cause the death of more normal cells than cancer cells. Almost all of the empirically derived, effective cancer therapies are directly toxic and inhibit vital processes such as DNA replication, protein synthesis, microtubule function, etc. Perhaps agents that inhibit, rather than cause, apoptosis might

be useful in conjunction with chemotherapy or radiotherapy to reduce dose-limiting side-effects due to apoptosis of normal cells. The p53 inhibitor pifithrin provides another approach to protect normal cells from apoptosis caused by cancer therapies (92,93). Used in conjunction with radiation or chemotherapy, this inhibitor would not provide any added advantage to *p53* null cancer cells, but might substantially reduce the side-effects. However, there is a theoretical risk that p53 inhibition might trigger a new malignancy elsewhere.

Harnessing our understanding of apoptosis in the treatment of cancer

A new understanding of radiation, chemotherapy and steroids

Most of the anticancer agents we use today were developed well before apoptosis became a fashionable subject or its mechanisms started to be unravelled and the most advanced novel therapies based on our understanding of apoptosis are still only at the clinical trial stage. Nevertheless, elucidation of the mechanisms of apoptosis and the pathways that regulate it have provided some new ways of looking at old therapies.

Both *in vitro* and *in vivo* evidence indicates that most if not all chemotherapies and radiotherapies can induce apoptosis of cancer cells, but also have direct toxic activities. The great majority induce apoptosis indirectly, as a response to the stress caused by their interference with an intracellular metabolic pathway. One or two established drugs, such as the steroid dexamethasone, are not intrinsically toxic, but directly induce apoptosis of cancer cells. It is to be expected that such agents would rapidly select subsets of tumor cells that have lost components of their apoptotic mechanism and, therefore, are resistant. Acting alone, these drugs would therefore not be likely to be curative, whereas drugs that are intrinsically toxic and block a vital metabolic pathway should kill cells even if the cells are incapable of an apoptotic response.

Death receptor ligands

Although TNF is not a useful anti-neoplastic agent clinically, in certain animal models it can cause death of tumor cells. It is now clear that it does not act directly on the cancer cells, but acts on the host endothelial cells required to feed the tumor (94). It might, therefore, be possible to use apoptosis to treat cancers indirectly, by interfering with vasculogenesis. Ligands of other death receptors, such as TRAIL (95), appear to work by direct induction of apoptosis of tumor cells, so to avoid selection of cells that have lost TRAIL receptors it will most likely be used in conjunction with chemotherapy.

Bcl-2 antagonists

Several approaches are being used to promote apoptosis of cancer cells by antagonizing Bcl-2 family members or by reducing their levels. A drug based on an antisense nucleotide to *bcl-2* is being trialed in a wide range of different cancers (96). Bcl-2/Bcl-x antagonists designed to mimic BH3-only peptides are being developed by a number of groups (97–100). These agents could be used to treat follicular lymphoma, in which elevated Bcl-2 levels contribute to causing the disease, but also in combination with conventional chemotherapeutic agents to test whether lowering Bcl-2 or Bcl-x will increase the proportion of tumor cells of a variety of types to undergo apoptosis.

Smac/Diablo mimetics

In *Drosophila*, IAPs are antagonized by Grim, HID, Reaper and Sickle, small cytoplasmic proteins that have similar N-termini by which they bind to the IAPs. Mammals have a number of mitochondrial proteins with similar N-termini that can bind to IAPs in the cytoplasm once they are released from the mitochondria. *In vitro* small peptides corresponding to the N-terminus of these proteins have been shown to be able to antagonize caspase inhibition by XIAP, and these are being used as the basis for development of IAP antagonist therapeutics (101–103).

Herceptin, Gleevec and Iressa

A number of agents have recently been developed that do not act directly on the cell death effector mechanism but block growth and survival signalling further upstream. Herceptin inhibits the epidermal growth factor receptor-2 plasma membrane tyrosine kinase (HER2/neu), whose expression is elevated by gene amplification in many breast cancers (104). Iressa inhibits signalling by the epidermal growth factor receptor (EGF-R) and has been trialed for treatment of lung cancer (105). The agent that has been most successful is Gleevec, which inhibits the Abl tyrosine kinase that is activated by the Philadelphia translocations in CML (106). These agents probably cause the same effects as removing factor-dependent cells from a source of growth factor, both stopping cells from cycling as well as causing default activation of the apoptosis mechanism. Which of these effects is most important therapeutically is not known.

Rituximab

One of the most promising agents for treatment of non-Hodgkin's lymphoma is a monoclonal antibody against the B cell antigen B220 (Rituximab). How this antibody leads to the death of lymphoma cells is not known, but proposed mechanisms include induction of apoptosis, activation of antibody-dependent cellular cytotoxicity or complement-mediated lysis (107).

p53–MDM2 complex

Novel agents have been developed that bind to MDM2, displacing p53 and thereby activating the p53 pathway, leading to cell cycle arrest and apoptosis (108). Such agents would have the advantage of not affecting normal cells and would be expected to work well against tumors overexpressing MDM2, but might rapidly select cancer cells that had lost or mutated p53.

Conclusions

It is now clear that frustration of the normal cell suicide process is an important and frequent contributory factor in the development of cancer. However, the significance of induction of apoptosis in cancer treatment remains uncertain. Although apoptosis of cancer cells is desirable (the only good cancer cell is a dead cancer cell) and many established and novel cancer therapies cause apoptosis both *in vitro* and *in vivo*, we still do not know how important apoptosis is compared with direct toxicity. We also do not know to what extent expression of apoptosis inhibitory genes contributes to resistance to cancer therapy. However, rapid progress in the field of apoptosis and the novel agents at all stages of development are grounds for optimism.

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