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Apoptosis and cancer: insights molecular mechanisms and treatments

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Abstract

Apoptosis is a form of cell death that permits the removal of damaged, senescent or unwanted cells in multicellular organisms, without damage to the cellular microenvironment, but it is also involved in a wide range of pathological processes, including cancer. An understanding of the underlying mechanism of apoptosis is important as it plays a pivotal role in the pathogenesis of many diseases. Defective apoptosis represents a major causative factor in the development and progression of cancer. The majority of chemotherapeutic agents, as well as radiation, utilize the apoptotic pathway to induce cancer cell death. Recent knowledge on apoptosis has provided the basis for novel targeted therapies that exploit apoptosis to treat cancer by acting in the extrinsic/intrinsic pathway. Defects can occur at any point along these pathways, leading to malignant transformation of the affected cells, tumour metastasis and resistance to anticancer drugs. In particular, this review provides references concerning the apoptotic molecules, their interactions, the mechanisms involved in apoptosis resistance, and also the modulation of apoptosis for the treatment of cancer. Despite being the cause of problem, apoptosis plays an important role in the treatment of cancer as it is a popular target of many treatment strategies.

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Introduction

Cell death, particularly apoptosis, is probably one of the most widely-studied subjects among cell biologists. Understanding apoptosis in disease conditions is very important as it not only gives insights into the pathogenesis of a disease but may also leaves clues on how the disease can be treated. In cancer, there is a loss of balance between cell division and cell death and cells that should have died did not receive the signals to do so. The problem can arise in any one step along the way of apoptosis. One example is the downregulation of p53, a tumour suppressor gene, which results in reduced apoptosis and enhanced tumour growth and development (Bauer and Hefand, 2006) and inactivation of p53, regardless of the mechanism, has been linked to many human cancers (Gasco *et al.,* 2002; Rodrigues *et al.,* 1990; Morton *et al.,* 2010). However, being a double-edged sword, apoptosis can be cause of the problem as well as the solution, as many have now ventured into the quest of new drugs targeting various aspects of apoptosis (Jensen *et al.,* 2008; Baritaki *et al.,* 2011). Hence, apoptosis plays an important role in both carcinogenesis and cancer treatment. This article gives a comprehensive review of apoptosis, its mechanisms, how defects along the apoptotic pathway contribute to carcinogenesis and how apoptosis can be used as a vehicle of targeted treatment in cancer.

Apoptosis

Apoptosis remains one of the most investigated processes in biologic research (Kerr *et al.,* 1972). Being a highly selective process, apoptosis is important in both physiological and pathological conditions (Mohan, 2010; Merkle, 2009).

Morphological changes in apoptosis

Morphological alterations of apoptotic cell death that concern both the nucleus and the cytoplasm are remarkably similar across cell types and species (Hacker, 2000; Saraste and Pulkki, 2000). Morphological hallmarks of apoptosis in the nucleus are chromatin condensation and nuclear fragmentation, which are accompanied by rounding up of the cell, reduction in cellular volume (pyknosis) and retraction of pseudopodes (Kroemer *et al.,* 2005). Chromatin condensation starts at the periphery of the nuclear membrane, forming a crescent or ring-like structure. The chromatin further condenses until it breaks up inside a cell with an intact membrane, a feature described as karyorrhexis (Manjo and Joris, 1995). At the later stage of apoptosis some of the morphological features include membrane blebbing, ultrastrutural modification of cytoplasmic organelles and a loss of membrane integrity (Kroemer *et al.,* 2005). Usually phagocytic cells engulf apoptotic cells before apoptotic bodies occur. However, the time taken depends on the cell type, the stimulus and the apoptotic pathway (Ziegler and Groscurth, 2004).

Biological changes in apoptosis

Biological cal changes can be observed in apoptosis: activation of caspases, DNA and protein breakdown, and membrane changes and recognition by phagocytic cells (Kumar *et al.,* 2010). Early in apoptosis, there is expression of phosphatidylserine (PS) in the outer layers of the cell membrane, which has been "flipped out" from the inner layers. This allows early recognition of dead cells by macrophages, resulting in phagocytosis without the release of pro-inflammatory cellular components (Hengartner, 2000). Later, there is internucleosomal cleavage of DNA into oligonucleosomes in multiples of 180 to 200 base pairs by endonucleases. Although this feature is characteristic of apoptosis, it is not specific as the typical DNA ladder in agarose gel electrophoresis can be seen in necrotic cells as well (McCarthy and Evan, 1998). Another specific feature of apoptosis is the activation of a group of enzymes belonging to the cysteine protease family named caspases. The "c" of "caspase" refers to a cysteine protease, while the "aspase" refers to the enzyme's unique property to cleave after aspartic acid residues (Kumar et al., 2010). Activated caspases cleave many vital cellular proteins

and break up the nuclear scaffold and cytoskeleton. They also activate DNAase, which further degrade nuclear DNA (Lavrik et al., 2005).

Molecular mechanisms of apoptosis

Understanding the mechanisms of apoptosis is crucial and helps in the understanding of the pathogenesis of conditions as a result of disordered apoptosis. This in turn, may help in the development of drugs that target certain apoptotic genes or pathways. Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners. There are three pathways by which caspases can be activated. The two commonly described initiation pathways are the intrinsic (or mitochondrial) and extrinsic (or death receptor) pathways of apoptosis (Fig. 1). Both pathways eventually lead to a common pathway or the execution phase of apoptosis. A third less well-known initiation pathway is the intrinsic endoplasmic reticulum pathway (O'Brien and Kirby, 2008).

Receptor mediated pathway:

The extrinsic death receptor pathway, as its name implies, begins when death ligands bind to a death receptor. Although several death receptors have been described, the best known death receptors is the type 1 TNF receptor (TNFR1) and a related protein called Fas (CD95) and their ligands are called TNF and Fas ligand (FasL) respectively (Hengartner, 2000). These death receptors have an intracellular death domain that recruits adapter proteins such as TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD), as well as cysteine proteases like caspase 8 (Schneider and Tschopp, 2000). Binding of the death ligand to the death receptor results in the formation of a binding site for an adaptor protein and the whole ligand-receptor-adaptor protein complex is known as the death-inducing signalling complex (DISC) (O'Brien and Kirby, 2008). DISC then initiates the assembly and activation of pro-caspase 8. The activated form of the enzyme, caspase 8 is an initiator caspase, which initiates apoptosis by cleaving other downstream or executioner caspases (Karp, 2008) (Fig. 1).

Mitochondrial pathway:

Regardless of the stimuli, this pathway is the result of increased mitochondrial permeability and the release of pro-apoptotic molecules such as cytochrome-c into the cytoplasm (Danial and Korsmeyer, 2004). This pathway is closely regulated by a group of proteins belonging to the Bcl-2 family (Tsujimoto et al., 1984). There are two main groups of the Bcl-2 proteins, namely the pro-apoptotic proteins (e.g. Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim and Hrk) and the anti-apoptotic proteins (e.g. Bcl-2, Bcl-X_L, Bcl-W, Bfl-1 and Mcl-1) (Reed, 1997). While the anti-apoptotic proteins regulate apoptosis by blocking the mitochondrial release of cytochrome-c, the pro-apoptotic proteins act by promoting such release. It is not the absolute quantity but rather the balance between the pro- and anti-apoptotic proteins that determines whether apoptosis would be initiated (Reed, 1997). Other apoptotic factors that are released from the mitochondrial intermembrane space into the cytoplasm include apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase (Smac), direct IAP Binding protein with Low pI (DIABLO) and Omi/high temperature requirement protein A (HtrA2) (Kroemer *et al.,* 2007). Cytoplasmic release of cytochrome c activates caspase 3 via the formation of a complex known as apoptosome which is made up of cytochrome c, Apaf-1 and caspase 9 (Kroemer *et al.,* 2007). On the other hand, Smac/DIABLO or Omi/HtrA2 promotes caspase activation by binding to inhibitor of apoptosis proteins (IAPs) which subsequently leads to disruption in the interaction of IAPs with caspase-3 or -9 (Kroemer *et al.,* 2007; LaCasse *et al.,* 2008) (Fig. 1).

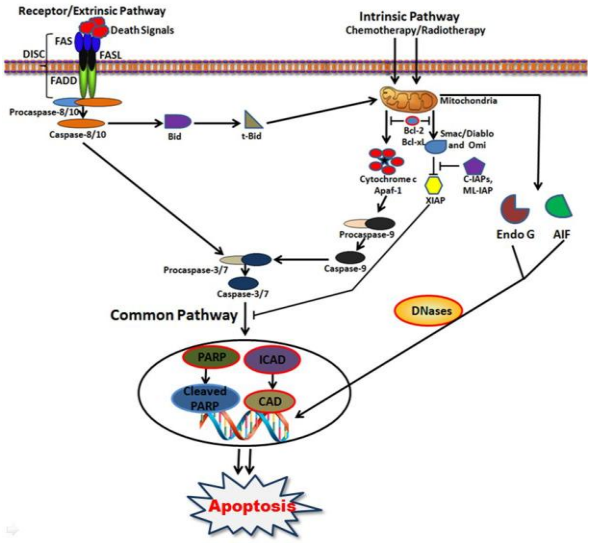

Fig 1. Apoptosis pathways. Death receptor pathway (left) is initiated by the ligation of the ligands to their respective surface receptors. Ligation of death receptors is followed by the formation of the death-inducible signalling complex (DISC), which results in the activation of pro-caspase-8/10 which cleaves caspase-8/10 and activates pro-caspase-3/7. The intrinsic pathway (right) is activated by death signals function directly or indirectly on the mitochondria, resulting in the formation of the apoptosome complex. This cell death pathway is controlled by Bcl-2 family proteins (regulation of cytochrome *c* release), inhibitor of apoptosis proteins (IAPs) and second mitochondrial activator of caspases (Smac/Omi). The intrinsic pathway might also operate through caspase-independent mechanisms, which involve the release from mitochondria and translocation to the nucleus of at least two proteins, apoptosis inducing factor (AIF) and endonuclease G (EndoG).

Common pathway:

The execution phase of apoptosis involves the activation of a series of caspases. The upstream caspase for the intrinsic pathway is caspase 9 while that of the extrinsic pathway is caspase 8. The intrinsic and extrinsic pathways converge to caspase 3. Caspase 3 then cleaves the inhibitor of the caspase-activated deoxyribonuclease (ICAD), which is responsible for nuclear apoptosis (Ghobrial *et al.*, 2005) (Fig. 1). In addition, downstream caspases induce cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins (PARP) and inhibitory subunits of endonucleases family. They also have an effect on the cytoskeleton, cell cycle and signalling pathways, which together contribute to the typical morphological changes in apoptosis (Ghobrial *et al.*, 2005).

Apoptosis and cancer

Cancer can be viewed as the result of a succession of genetic changes during which a normal cell is transformed into a malignant one while evasion of cell death is one of the essential changes in a cell that cause this malignant transformation (Hanahan, 2000). Hence, reduced apoptosis or its resistance plays a vital role in carcinogenesis. There are many ways a malignant cell can acquire reduction in apoptosis or apoptosis resistance. Generally, the mechanisms by which evasion of apoptosis occurs can be broadly dividend into: disrupted balance of pro-apoptotic and anti-apoptotic proteins, reduced caspase function, and impaired death receptor signalling. Fig. 2 summarises the mechanisms that contribute to evasion of apoptosis and carcinogenesis.

Imbalance of pro- and anti-apoptotic proteins

Many proteins have been reported to exert pro- or anti-apoptotic activity in the cell. It is not the absolute quantity but rather the ratio of these pro-and anti-apoptotic proteins that plays an important role in the regulation of cell death. Besides, over- or under-expression of certain genes (hence the resultant regulatory proteins) have been found to contribute to carcinogenesis by reducing apoptosis in cancer cells.

Bcl-2 family proteins:

Bcl-2 family proteins is comprised of pro-apoptotic and anti-apoptotic proteins that play a pivotal role in the regulation of apoptosis, especially via the intrinsic pathway as they reside upstream of irreversible cellular damage and act mainly at the mitochondria level

(Gross, 1999). Bcl-2 was the first protein of this family to be identified more than 20 years ago and it is encoded by the BCL2 gene, which derives its name from B-cell lymphoma 2, the second member of a range of proteins found in human B-cell lymphomas with the t (14; 18) chromosomal translocation (Tsujimoto *et al.,* 1984). All the Bcl-2 members are located on the outer mitochondrial membrane. They are dimmers which are responsible for membrane permeability either in the form of an ion channel or through the creation of pores (Minn *et al.,* 1997). Based of their function and the Bcl-2 homology (BH) domains the Bcl-2 family members are further divided into three groups (Dewson and Kluc, 2010). The first groups are the anti-apoptotic proteins that contain all four BH domains and they protect the cell from apoptotic stimuli. Some examples are Bcl-2, Bcl-xL, Mcl-1, Bcl-w, A1/Bfl-1, and Bcl-B/Bcl2L10. The second group is made up of the BH-3 only proteins, so named because in comparison to the other members, they are restricted to the BH3 domain. Examples in this group include Bid, Bim, Puma, Noxa, Bad, Bmf, Hrk, and Bik. In times of cellular stresses such as DNA damage, growth factor deprivation and endoplasmic reticulum stress, the BH3-only proteins, which are initiators of apoptosis, are activated. Therefore, they are pro-apoptotic. Members of the third group contain all four BH domains and they are also pro-apoptotic. Some examples include Bax, Bak, and Bok/Mtd (Dewson and Kluc, 2010). When there is disruption in the balance of anti-apoptotic and pro-apoptotic members of the Bcl-2 family, the result is dysregulated apoptosis in the affected cells. This can be due to an overexpression of one or more anti-apoptotic proteins or an underexpression of one or more pro-apoptotic proteins or a combination of both. For example, Raffo *et al* showed that the overexpression of Bcl-2 protected prostate cancer cells from apoptosis (Raffo *et al.,* 1995) while Bcl-2 overexpression led to inhibition of TRAIL-induced apoptosis in neuroblastoma, glioblastoma and breast carcinoma cells (Fulda *et al.,* 2000). Overexpression of Bcl-xL has also been reported to confer a multi-drug resistance phenotype in tumour cells and prevent them from undergoing apoptosis (Minn *et al.,* 1995). In colorectal cancers with microsatellite instability, on the other hand, mutations in the *bax* gene are very common. Miquel *et al* demonstrated that impaired apoptosis resulting from*bax* (G)8 frameshift mutations could contribute to resistance of colorectal cancer cells to anticancer treatments (Miquel *et al., 2005*). In the case of chronic lymphocytic leukaemia (CLL), the malignant cells have an anti-apoptotic phenotype with high levels of anti-apoptotic Bcl-2 and low levels of pro-apoptotic proteins such as Bax *in vivo*. Leukaemogenesis in CLL is due to reduced apoptosis rather than increased proliferation *in vivo (*Goolsby *et al.,* 2005*)*. Pepper *et al* reported that B-lymphocytes in CLL showed an increased Bcl-2/Bax ratio in patients with CLL and that when these cells were cultured *in vitro*, drug-induced apoptosis in B-CLL cells was inversely related to Bcl-2/Bax ratios (Pepper *et al.,* 1997).

Tumour suppressor p53 proteins:

The p53 protein, also called tumour protein 53, is one of the best known tumour suppressor proteins encoded by the tumour suppressor gene *TP53* located at the short arm of chromosome 17 (17p13.1). It is named after its molecular weights, i.e., 53 kDa (Levine *et al.,* 1991). It is not only involved in the induction of apoptosis but it is also a key player in cell cycle regulation, development, differentiation, gene amplification, DNA recombination, chromosomal segregation and cellular senescence (Oren and Rotter, 1999). Defects in the p53 tumour suppressor gene have been linked to more than 50% of human cancers (Bai and Zhu, 2006). Recently, Avery-Kieida *et al.* reported that some target genes of p53 involved in apoptosis and cell cycle regulation are aberrantly expressed in melanoma cells, leading to abnormal activity of p53 and contributing to the proliferation of these cells (Avery-Kiejda *et al.,* 2011). In addition, it has been found that when the p53 mutant was silenced, such down-regulation of mutant p53 expression resulted in

reduced cellular colony growth in human cancer cells, which was found to be due to the induction of apoptosis (Vikhanskaya *et al.,* 2007).

Inhibitor of apoptosis proteins (IAPs):

The inhibitor of apoptosis proteins are a group of structurally and functionally similar proteins that regulate apoptosis, cytokinesis and signal transduction. To date eight IAPs have been identified, namely, NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/ML-IAP (BIRC7) and IAP-like protein 2 (BIRC8) (Vucic and Fairbrother, 2007). IAPs are endogenous inhibitors of caspases and they can inhibit caspase activity by binding their conserved BIR domains to the active sites of caspases, by promoting degradation of active caspases or by keeping the caspases away from their substrates (Wei *et al.,* 2008). Dysregulated IAP expression has been reported in many cancers. For example, Lopes *et al* demonstrated abnormal expression of the IAP family in pancreatic cancer cells and that this abnormal expression was also responsible for resistance to chemotherapy. Another IAP, Survivin, has been reported to be overexpressed in various cancers. Small *et al.* observed that transgenic mice that overexpressed Survivin in haematopoietic cells were at an increased risk of haematological malignancies and that haematopoietic cells engineered to overexpress Survivin were less susceptible to apoptosis (Small *et al.,* 2010). Survivin, together with XIAP, was also found to be overexpressed in non-small cell lung carcinomas (NSCLCs) and the study concluded that the overexpression of Survivin in the majority of NSCLCs together with the abundant or upregulated expression of XIAP suggested that these tumours were endowed with resistance against a variety of apoptosis-inducing conditions (Krepela et al., 2009).

Decreased capsase activity

The caspases can be broadly classified into two groups: those related to caspase 1 (e.g. caspase-1, -4, -5, -13, and -14) and are mainly involved in cytokine processing during inflammatory processes and those that play a central role in apoptosis (e.g. caspase-2, -3. -6, -7,-8, -9 and -10). The second group can be further classified into initiator caspases (e.g. caspase-2, -8, -9 and -10) which are primarily responsible for the initiation of the apoptotic pathway and effector caspases (caspase-3, -6 and -7) which are responsible in the actual cleavage of cellular components during apoptosis (Fink *et al.,* 2005). It is therefore reasonable to believe that low levels of caspases or impairment in caspase function may lead to a decreased in apoptosis and carcinogenesis.

Disregulation of death receptor signalling

Death receptors and ligands of the death receptors are key players in the extrinsic pathway of apoptosis. Other than TNFR1 (also known as DR 1) and Fas (also known as DR2, CD95 or APO-1) mentioned in Section 2.3, examples of death receptors include DR3 (or APO-3), DR4 [or TNF-related apoptosis inducing ligand receptor 1 (TRAIL-1) or APO-2], DR5 (or TRAIL-2), DR 6, ectodysplasin A receptor (EDAR) and nerve growth factor receptor (NGFR) (Lavrik *et al.,* 2005). These receptors posses a death domain and when triggered by a death signal, a number of molecules are attracted to the death domain, resulting in the activation of a signalling cascade. However, death ligands can also bind to decoy death receptors that do not possess a death domain and the latter fail to form signalling complexes and initiate the signalling cascade (Lavrik *et al.,* 2005).

Targeting apoptosis in cancer treatment

Drugs or treatment strategies that can restore the apoptotic signalling pathways towards normality have the potential to eliminate cancer cells, which depend on these defects to stay alive. Many recent and important discoveries have opened new doors into potential new classes of anticancer drugs.

Bcl-2 family proteins

Some potential treatment strategies used in targeting the Bcl-2 family of proteins include the use of therapeutic agents to inhibit the Bcl-2 family of anti-apoptotic proteins or the silencing of the upregulated anti-apoptotic proteins or genes involved.

Drug that effect on Bcl-2 family proteins:

One good example of these agents is the drug oblimersen sodium, which is a Bcl-2 antisence oblimer, the first agent targeting Bcl-2 to enter clinical trial. The drug has been reported to show chemosensitising effects in combined treatment with conventional anticancer drugs in chronic myeloid leukaemia patients and an improvement in survival in these patients (Rai et al., 2008; Abou-Nassar and Brown, 2010), examples include sodium butyrate, depsipetide, fenretinide, flavipirodol, gossypol, ABT-737, ABT-263, GX15-070 and HA14-1(Kang and Reynolds, 2009). Some of these small molecules belong to yet another class of drugs called BH3 mimetics, so named because they mimic the binding of the BH3-only proteins to the hydrophobic groove of anti-apoptotic proteins of the Bcl-2 family. One classical example of a BH3 mimetic is ABT-737, which inhibits anti-apoptotic proteins such as Bcl-2, Bcl-xL, and Bcl-W. It was shown to exhibit cytotoxicity in lymphoma, small cell lung carcinoma cell line and primary patient-derived cells and caused regression of established tumours in animal models with a high percentage of cure (Oltersdorf *et al.,* 2005). Other BH3 mimetics such as ATF4, ATF3 and NOXA have been reported to bind to and inhibit Mcl-1 (Albershardt *et al.,* 2011).

Inactvating the anti-apoptotic proteins/genes:

Rather than using drugs or therapeutic agents to inhibit the anti-apoptotic members of the Bcl-2 family, some studies have demonstrated that by silencing genes coding for the Bcl-2 family of anti-apoptotic proteins, an increase in apoptosis could be achieved. For example, the use of Bcl-2 specific siRNA had been shown to specifically inhibit the expression of target gene *in vitro* and *in vivo* with anti-proliferative and pro-apoptotic effects observed in pancreatic carcinoma cells (Ocker *et al.,* 2005). On the other hand, silencing Bmi-1 in MCF breast cancer cells, the expression of pAkt and Bcl-2 was downregulated, rendering these cells more sensitive to doxorubicin as evidenced by an increase in apoptotic cells *in vitro* and *in vivo (*Wu *et al.,* 2011*).*

Molecular target of p53

Many p53-based strategies have been investigated for cancer treatment. Generally, these can be classified into three broad categories: gene therapy, drug therapy, and immunotherapy.

p53-dependent gene therapy:

The first report of p53 gene therapy in 1996 investigated the use of a wild-type p53 gene containing retroviral vector injected into tumour cells of non-small cell lung carcinoma derived from patients and showed that the use of p53-based gene therapy may be feasible (Roth *et al.,* 1996). As the use of the p53 gene alone was not enough to eliminate all tumour cells, later studies have investigated the use of p53 gene therapy concurrently with other anticancer strategies. For example, the introduction of wild-type p53 gene has been shown to sensitise tumour cells of head and neck, colorectal and prostate cancers and glioma to ionising radiation (Chène, 2001). Although a few studies managed to go as far as phase III clinical trials, no final approval from the FDA has been granted so far (Suzuki and Matusubara, 2011). Another interesting p53 gene-based strategy was the use of engineered viruses to eliminate p53-deficient cells. One such example is the use of a genetically engineered oncolytic adenovirus, ONYX-015, in which the *E1B-55 kDa* gene has been deleted, giving the virus the ability to selectively replicate in and lyse tumour cells deficient in p53 (John *et al.,* 2000).

p53-mediated drug therapy:

Several drugs have been investigated to target p53 via different mechanisms. One class of drugs is small molecules that can restore mutated p53 back to their wild-type functions. For example, Phikan083, a small molecule and carbazole derivative, has been shown to bind to and restore mutant p53 (Boeckler *et al.,* 2008). Another small molecule, CP-31398, has been found to intercalate with DNA and alter and destabilise the DNA-p53 core domain complex, resulting in the restoration of unstable p53 mutants (Rippin et al., 2002). Other drugs that have been used to target p53 include the nutlins, MI-219 and the tenovins. Nutlins are analogues of cis-imidazoline, which inhibit the MSM2-p53 interaction, stabilise p53 and selectively induce senescence in cancer cells (Shangary and Wang, 2008) while MI-219 was reported to disrupt the MDM2-p53 interaction, resulting in inhibition of cell proliferation, selective apoptosis in tumour cells and complete tumour growth inhibition (Shangary *et al.,* 2008). The tenovins, on the other hand, are small molecule p53 activators, which have been shown to decrease tumour growth *in vivo (*Lain *et al.,* 2008*).*

p53-based immunotherapy:

Several clinical trials have been carried out using p53 vaccines. In a clinical trial by six patients with advanced-stage cancer were given vaccine containing a recombinant replication-defective adenoviral vector with human wild-type p53. When followed up at 3 months post immunisation, four out of the six patients had stable disease. However, only one patient had stable disease from 7 months onwards (Kuball *et al.,* 2002). Other than viral-based vaccines, dendritic-cell based vaccines have also been attempted in clinical trials. The use of p53 peptide pulsed dendritic cells in a phase I clinical trial and reported a clinical response in two out of six patients and p53-specific T cell responses in three out of six patients (Svane *et al.,* 2004). Other vaccines that have been used including short peptide-based and long peptide-based vaccines (Vermeij *et al.,* 2011).

Recent target of IAPs and Survivin

When designing novel drugs for cancers, the IAPs are attractive molecular targets. So far, XIAP has been reported to be the most potent inhibitor of apoptosis among all the IAPs. It effectively inhibits the intrinsic as well as extrinsic pathways of apoptosis and it does so by binding and inhibiting upstream caspase-9 and the downstream caspases-3 and -7 (Dai *et al.,* 2009). Some novel therapy targeting XIAP include antisense strategies and short interfering RNA (siRNA) molecules. Using the antisense approach, inhibition of XIAP has been reported to result in an improved *in vivo*tumour control by radiotherapy (Cao *et al.,* 2004). When used together with anticancer drugs XIAP antisense oligonucleotides have been demonstrated to exhibit enhanced chemotherapeutic activity in lung cancer cells *in vitro* and *in vivo (*Hu *et al.,* 2003*)*. On the other hand, siRNA targeting of XIAP increased radiation sensitivity of human cancer cells independent of TP53 status (Ohnishi *et al.,* 2006) while targeting XIAP or Survivin by siRNAs sensitise hepatoma cells to death receptor- and chemotherapeutic agent-induced cell death (Yamaguchi *et al.,* 2005).

Survivin is highly expressed during embryo development whereas it is more or less absent in a large number of normal differentiated tissues (Ambrosini *et al.,* 2002). Many studies have investigated various approaches targeting Survivin for cancer intervention. One example is the use of antisense oligonucleotides. Grossman *et al* was among the first to demonstrate the use of the antisense approach in human melanoma cells. It was shown that transfection of anti-sense Survivin into YUSAC-2 and LOX malignant melanoma cells resulted in spontaneous apoptosis in these cells (Grossman *et al.,* 1999). The anti-sense approach has also been applied in head and neck squamous cell carcinoma and reported to induce apoptosis and sensitise these cells to chemotherapy (Sharma *et al.,* 2005) and in medullary thyroid carcinoma cells, and was found to inhibit growth and proliferation of these cells (Du et

al., 2006). Another approach in targeting Survivin is the use of siRNAs, which have been shown to downregulate Survivin and diminish radioresistance in pancreatic cancer cells (Kami *et al.,* 2005), to inhibit proliferation and induce apoptosis in SPCA1 and SH77 human lung adenocarcinoma cells (Liu et al., 2011), to suppress Survivin expression, inhibit cell proliferation and enhance apoptosis in SKOV3/DDP ovarian cancer cells (Zhang *et al.,* 2009) as well as to enhance the radiosensitivity of human non-small cell lung cancer cells (Yang *et al.,* 2010). Besides, small molecules antagonists of Survivin such as cyclin-dependent kinase inhibitors and Hsp90 inhibitors and gene therapy have also been attempted in targeting Survivin in cancer therapy (Pennati *et al*., 2007). Other IAP antagonists include peptidic and non-peptidic small molecules, which act as IAP inhibitors. Two cyclopeptidic Smac mimetics, 2 and 3, which were found to bind to XIAP and cIAP-1/2 and restore the activities of caspases- 9 and 3/-7 inhibited by XIAP were amongst the many examples (Sun *et al.,* 2010). On the other hand, SM-164, a non-peptidic IAP inhibitor was reported to strongly enhance TRAIL activity by concurrently targeting XIAP and cIAP1 (Lu *et al.,* 2011).

Important targeting of caspases

Several drugs have been designed to synthetically activate caspases. For example, Apoptin is a caspase-inducing agent which was initially derived from chicken anaemia virus and had the ability to selectively induce apoptosis in malignant but not normal cells (Rohn, 2004). Another class of drugs which are activators of caspases are the small molecules caspase activators. These are peptides which contain the arginin-glycine-aspartate motif. They are pro-apoptotic and have the ability to induce auto-activation of procaspase 3 directly. They have also been shown to lower the activation threshold of caspase or activate caspase, contributing to an increase in drug sensitivity of cancer cells (Philchenkov *et al.,* 2004). In addition to caspase-based drug therapy, caspase-based gene therapy has been attempted in several studies. For instance, human caspase-3 gene therapy was used in addition to etoposide treatment in an AH130 liver tumour model and was found to induce extensive apoptosis and reduce tumour volume (Yamabe *et al.,* 1999) while gene transfer of constitutively active caspse-3 into HuH7 human hepatoma cells selectively induced apoptosis in these cells (Cam *et al.,* 2005). Also, a recombinant adenovirus carrying immunocaspase 3 has been shown to exert anti-cancer effects in hepatocellular carcinoma *in vitro* and *in vivo (*Li *et al.,* 2007*).*

Preclinical studies

Pre-clinical in vitro studies on prostate and lung tumor cells have reported synergic effects when exisulind is used together with docetaxel or paclitaxel (Soriano *et al.,* 1999), probably because both drugs lead to JNK activation and to the promotion of apoptosis. In the murine model it has been demonstrated that exisulind, like sulindac, is able to inhibit the growth of several tumor cells, for example, of the colon, prostate, bladder and breast (Thompson *et al.,* 1997), prostate (Goluboff *et al.,* 1999) and lung (Malkinson *et al.,* 1998). Unlike sulindac, however, exisulind does not inhibit Cox-1 and Cox-2 activity. The results obtained from the various *in vivo* and *in vitro* studies have shown that survivin inhibition not only increases the efficiency of traditional chemotherapy drugs, but that it is also able to reduce tumoral angiogenesis (Nicholson, 2000).

Clinical studies

Several clinical studies, either already concluded or still in progress, have shown that exisulind, because of its tolerability and activity, could be used for the treatment of solid tumors such as for prostate tumors (Goluboff *et al.,* 2001). A recent phase I study has determined the maximal tolerated dose (MTD) of the combination of weekly docetaxel and exisulind in patients with advanced solid tumors (Garcia *et al.,* 2006). However, although preclinical data demonstrate increased apoptosis and prolonged

survival for the combination of exisulind and docetaxel, multiple clinical trials do not support further clinical development of this combination regimen in patients with advanced NSCLC (Jones *et al.,* 2005). Furthermore, in sporadic colonic adenomas, exisulind causes significant regression of sporadic adenomatous polyps but is associated with toxicity (Arber *et al.,* 2006). Clinical trials are also in progress at present on the use of antisense oligonuceleotides of survivin (Zaffaroni *et al.,* 2005).

Conclusions

The last decade has seen an extraordinary increase in our understanding of the complexities of apoptosis and the mechanisms evolved by tumor cells to resist engagement of cell death. The activation of alternative pathways by proapoptotic approaches such as death receptors (e.g. TRAIL) or the introduction of exogenous proapoptotic molecules such as apoptin are capable of inducing apoptosis even in a genetically altered context. Although at present there are still many components of the apoptotic pathways that are still not fully understood, the information collected so far has led to a better knowledge of the mechanisms of resistance to standard chemo-and radio-therapy, as well as possible strategies aimed at restoring apoptotic sensitivity. Furthermore, the genetic features of each individual tumor and apoptotic response will make it possible to choose a more suitable therapeutic approach with the aim of overcoming treatment resistance and limiting cytotoxic effects in normal tissues. Based on the present knowledge, the use of these 'biological drugs' in synergistic association with the traditional cytotoxic drugs might represent an important goal in the treatment of malignant cells and not the normal ones.

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