Apoptosis in Cancer Cell Signaling and Current Therapeutic Possibilities

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Abstract

Apoptosis is programmed cell death, which sustains the equilibrium between survival and death in eukaryotic cells. It is a tightly regulated cell death program that aims at eliminating harmful, damaged, or unwanted cells. This wisely programmed cell death is central in the development of all multicellular organisms, which is highlighted by the prevalence of diseases associated with abnormal apoptosis. For example, defect in apoptosis is a hallmark of cancer, whereas excessive cell death occurs in several neurodegenerative disorders. The cell death signals are responsible for maintenance of the genomic integrity, while defective cell death may stimulate carcinogenesis. These signals are convoluted and are controlled at various points. Tumor cells survive by taking help of several different molecular mechanisms to inhibit apoptosis and acquire resistance to apoptotic agents, for example, by the expression of anti-apoptotic proteins such as Bcl-2 or by the downregulation or mutation of pro-apoptotic proteins such as BAX. This chapter includes recent developments in the field and reviews new evidences of the interconnection between apoptosis and cancer. Various molecules that can be regulated to facilitate apoptosis in myriad of cancers are also enlisted. Overall, the chapter discusses about the development of various treatments and approaches to combat cancer by targeting anti-apoptotic proteins belonging to Bcl-2 and IAP families.

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5.1   Introduction

5.1.1   Apoptosis: A Programmed Cell Death

The average adult human body makes around 50–70 billion new cells every day by somatic cell division also called mitosis. Therefore, to maintain a constant number of cells in the body, they must also die a natural death. In humans, about a million of cells are made every second through mitosis, whereas a similar number is destroyed by the process of apoptosis – this is known as cellular homeostasis (Vaux and Korsmeyer 1999). The word "apoptosis" (Greek origin) is divided into two parts, *apo* (from) and *ptosis* (falling), that describes falling of leaves from a tree (Wong 2011). Including apoptosis, there are various types and subtypes of cell death. The primary ones are as follows:

1. *Necrosis*: Uncontrolled cell death. It is generally the outcome of cellular injury and/or infection. Phenotypically, the cells burst, emitting their contents into the surrounding tissue fluid; this stimulates inflammation, pain, and swelling.
2. *Apoptosis* (programmed cell death): It is a programmed, multistep pathway that leads to cell death for the normal functioning of cellular growth and tissue homeostasis. It is developed as an irreversible practice to proficiently remove nonfunctional and old cells (Letai 2008). There are stepwise events, such as enzyme activation, required for this (unlike necrosis). In apoptosis, genetic control is preserved till the end (Vo and Letai 2010).
3. *Autophagy:* It is a naturally occurring controlled mechanism by which a cell can dismantle its redundant and defective components in a systematic manner (Yu et al. 2004).

Generally, the morphological features of a dying cell decide the mode of cell death. Consequently, apoptosis has been designated as *type I* programmed cell death, autophagy as *type II*, and necrosis as a type of death that combines characteristics of both *type I* and *type II*. With the advent of new biochemical methods over the past few decades, a more conclusive categorization of cell death types has become possible. Cell death classifications have yielded categories which render to biochemical characteristics, morphological features, immunological facets, or functional references. Normally, cell cleaning process or apoptosis starts with breaking down of the genetic materials across the nuclear margins, followed by cell fragmentation and generation of cell debris, which in turn are consumed by phagocytes (Figs. 5.1 and 5.2). A series of biochemical events leading to apoptosis includes activation of proteases like caspases, cleavage of DNA and proteins, and exposure of phosphatidylserine on the surface of the cell. Along with elimination of unwanted cells and tissues, apoptosis also plays a major role in development of the embryo and neurologic pruning. Categorically, phases of apoptosis include the *initiation phase* and *execution phase* (Kroemer et al. 2007).
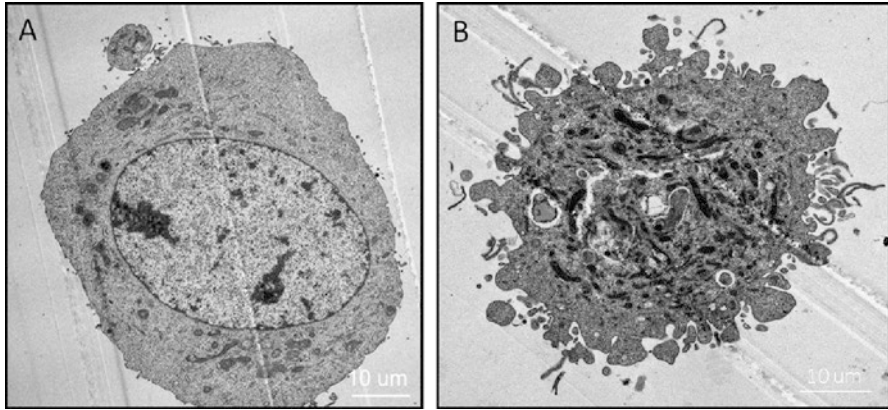
Fig. 5.1 Ultrastructural examination of typical apoptotic cell using transmission electron microscopy (TEM). Untreated control HEK293T cell. (**a**) exhibit normal cell morphology including normal cell nuclei, cytoplasm, and cell organelles. (**b**) Drug-treated cell indicates the appearance characteristics of apoptotic cell such as membrane blebbing, nuclear condensation, and damage to cell organelles. Magnification, x8,000. Image is under publication (*Bose Lab, unpublished results*)
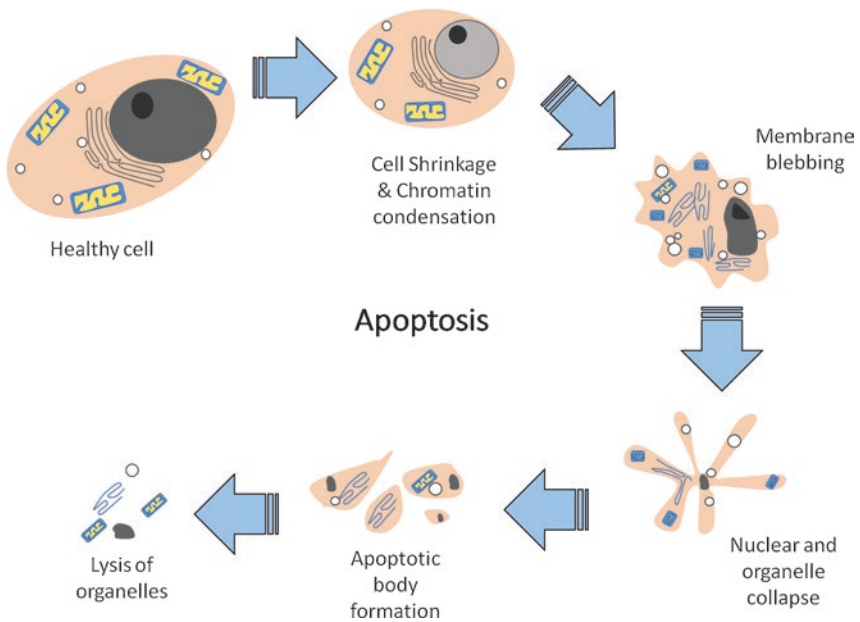
Fig. 5.2 Hallmarks of apoptotic cell death. Cartoon illustrating the basic stages of the apoptotic pathway. Apoptosis includes cellular shrinking, chromatin condensation, and margination at the nuclear periphery with the eventual formation of membrane-bound apoptotic bodies that contain organelles, cytosol, and nuclear fragments

5.1.2   Initiation Phase

This phase of apoptosis mostly involves a complex entanglement of a number of proteins. It depends on the cell type, apoptotic stimulus, and activation of proteases, nucleases, and other effector molecules. Normally, it gets activated by several intracellular and extracellular stresses (Bender and Martinou 2013). Extracellular signals that induce apoptosis include dysfunctioning growth factors, hypoxia, and environmental radiation. Intracellular signals include DNA damage, damage caused by chemotherapeutic drugs, telomere malfunction, and viral infections. The two different important pathways that mediate initiation phase are discussed in the following sections.

5.1.2.1 Extrinsic Pathway Mediated by Receptor

It is induced by external events of cells mediated by the death receptors. In this pathway, members of the tumor necrosis factor (TNF) receptor superfamily of transmembrane proteins are mainly involved. The most notable feature of TNF receptors is a region called "death domain" which roughly consists of 80 amino acids (Bradley and Pober 2001). Transferring death signals across cellular membrane is the most significant role of these death domain regions. Two most well-known death receptors are the type 1 TNF receptor (TNFR-1) and a related protein called Fas (CD95). Upon receiving apoptotic stimuli, receptors trimerize after binding to their respective extracellular ligands, TNF and Fas ligand (FasL) (Hengartner 2001). Post trimerization, these death receptors recruit adapter proteins such as TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD) through homotypic protein-protein interactions. Subsequently, the adaptor molecules bind to initiator cysteine proteases such as caspase 8/10 through similar homotypic interactions and that forms a complex called death inducing signaling complex (DISC) (Singh et al. 2016). DISC formation is a prerequisite of autoactivation of upstream or initiator caspases 8/10 with the subsequent activation of downstream caspases 3/7 (Wong 2011) that lead to initiation of the caspase cascade, hence apoptosis (Schneider and Tschopp 2000). Extrinsic cell death pathway is strongly controlled by relative expression of both pro-apoptotic and anti-apoptotic proteins, and a disproportion in that regulation leads to several diseases.

Defects in Extrinsic Pathway Signaling and Carcinogenesis

Genetic studies on mice in which certain genes encode death ligands or their receptors have given sufficient proof of role in tumor suppression by cellular immune mechanisms. Fas ligand (FasL) is essential when there is cytotoxic T lymphocytes (CTL)-mediated killing of tumor cells, and TRAIL/Apo2L (TNF-related apoptosis-inducing ligands) is important for natural killer (NK)-cells-mediated tumor suppression. However, a number of cancer cells escape immune annihilation by resisting the response of the death receptor pathway to FasL. Although numerous available cancer cell lines express various cell surface receptors, they show an inherent resistance to TRAIL. It somehow indicates that during the course of evolution of tumors in vivo, an enforced selection of malignant clones takes place, which are

capable of defying immune attack. Some of the examples are downregulation of the Fas receptor, abundance of nonfunctioning or precursor of Fas receptors, and generation of large amount of soluble Fas receptor. Moreover, some cancer cells can execute a "counterattack" facilitated by Fas ligand that results in apoptotic reduction of activated tumor-sensitive lymphocytes (Koyama et al. 2001). Thus, it is very important to have a promising therapeutic approach that can rely on making extrinsic pathway to act on nonessential cells.

5.1.2.2 Intrinsic Pathway or In-House (Mitochondria Dependent)

This pathway is initiated by internal cell death gestures, mainly severe nuclear DNA damage, low oxygen content in the cells, viral infection, etc. It is majorly controlled by proteins in the Bcl-2 (B-cell lymphoma 2) family, comprising a total of 25 known proteins. They are involved to either excite (pro-apoptotic) or inhibit apoptosis (anti-apoptotic) (Hengartner 2000).Under normal conditions, there is an equilibrium among pro-apoptotic and anti-apoptotic proteins present in the cell**.**

The BH3-only (Bcl-2 Homology-3) proteins when respond to signs, such as loss of genetic material to undergo apoptosis, position themselves to the mitochondrial outer membrane and trigger the two important proteins Bax or Bak. When stimulated, Bax and Bak heterodimer cause mitochondrial outer membrane permeabilization (MOMP) (Bender and Martinou 2013). This heterodimer penetrates the membrane and creates pores all over the membrane that help other apoptotic factors like cytochrome-c to come out into the cytosol. Later on, it binds to caspase 3 forming a crucial compound/complex known as apoptosome comprising cytochrome-c, Apaf-1 (actin filament-associated protein 1), and nonfunctional initiator caspase-9 (Kroemer et al. 2007). The newly formed composite structure works by hydrolyzing adenosine triphosphate (ATP) and form a functional caspase 9. The active/functional caspase 9 further reacts with the executioner caspases 3, 6, and 7 to carry out their respective functions that ultimately help in cellular apoptosis. This pathway is distinct from the one emanating from extracellular signals. Apart from them, some apoptotic factors like Apoptosis-inducing factor (AIF), second mitochondria-derived activator of caspase (SMAC), and Omi/high-temperature requirement protein A-2 (HtrA2) are also exuded out from the intermembrane space of mitochondria into the cytoplasm (Kroemer et al. 2007).

Defects in Intrinsic Pathway Signaling and Carcinogenesis

One of the key proteins found to be associated with intrinsic pathway of apoptosis is caspase 9, whose impairment leads to detrimental consequences. For example, caspase-9-knockout mice show juvenile death due to abnormalities in the brain structure/function due to accumulation of increased number of unnecessary cells (Hakem et al. 1998; Kuida et al. 1998). Furthermore, lymphocytes within the thymus glands from the knockout mice display susceptibility toward common variety of anticancer drugs and chemo-radiations (Kuida et al. 1998).

There are reports suggesting reduced expression of caspase 9 in the epithelial cells from colorectal cancer patient's cells related to cells from healthy individual (Hector et al. 2012). Apart from that, anti-apoptotic proteins (e.g., Bcl-2, Bcl-X_L

(B-cell lymphoma 2-Xetra large), Bcl-2 L2 (B-cell lymphoma 2 like protein 2), and Mcl-1 (induced myeloid leukemia cell differentiation protein)), which have conserved BH1–BH4 domains are known to promote cell survival, whereas proapoptotic proteins are associated with stress-dependent apoptosis. There is a second group of multidomain BH3-only proteins such as Bax and Bak, which are essential for apoptosis (Reed 2006). Anti-apoptotic proteins block apoptosis by counteracting the actions of Bax/Bak complex through a known mechanism (Green 2006). Overexpression of anti-apoptotic Bcl-2 or Bcl-X_L occurs in more than half of all cancers (Amundson et al. 2000) by rendering cancer cells resistant to apoptotic stimuli that also include majority of cytotoxic anticancer drugs.

5.1.2.3 Perforin/Granzymes

As a part of human cellular immunity, in response to several stress conditions, cells such as cytotoxic T lymphocytes can cause apoptosis. The procedure involves activation of such cells followed by secretion of special proteins called perforin and its associated enzymes. Their main job is to form pores onto the cell membrane of the target cell. The additional particles use these pores to enter the cell. After entering the cell, they release their enzymes (granzymes A and B) that start execution of apoptosis by causing destruction of cellular structure and function (Trapani and Smyth 2002).

5.1.3   Execution Phase

The initiation phase activates the execution phase. The execution phase involves the activation of a group of cysteine proteases named as caspases which plays cell execution (Wong 2011; Bender and Martinou 2013). These proteins are ubiquitously present in all cell types, and their expression is enough to carry out its function. Among all, caspase 3 is a well-known executioner caspase. Once this protein takes charge as an executioner, apoptosis is definite. It is activated in major functions mainly DNA and chromatin damage, cell division, as well as signal transduction.

5.2   Resistance to Apoptosis in Cancer

Uncontrolled proliferation of cells give birth to variety of cancers (King and Cidlowski 1998). Moreover, the role of malfunctioning apoptotic machineries during cancer metastasis is well documented in myriad of cancers (Kerr and Searle 1972). Normally, tumor cells use various molecular mechanisms to acquire resistance to apoptosis. These processes mostly include upregulation of anti-apoptotic proteins like Bcl-2 and downregulation of pro-apoptotic proteins such as BAX. As regulation of both proteins is controlled/regulated by the p53 gene (Miyashita et al. 1994), some forms of B-cell lymphoma show Bcl-2 overexpression (Fig. 5.3). Therefore, many similar examples represent the first line of evidences that failure of apoptosis contributes to cancer (Vaux 1998).
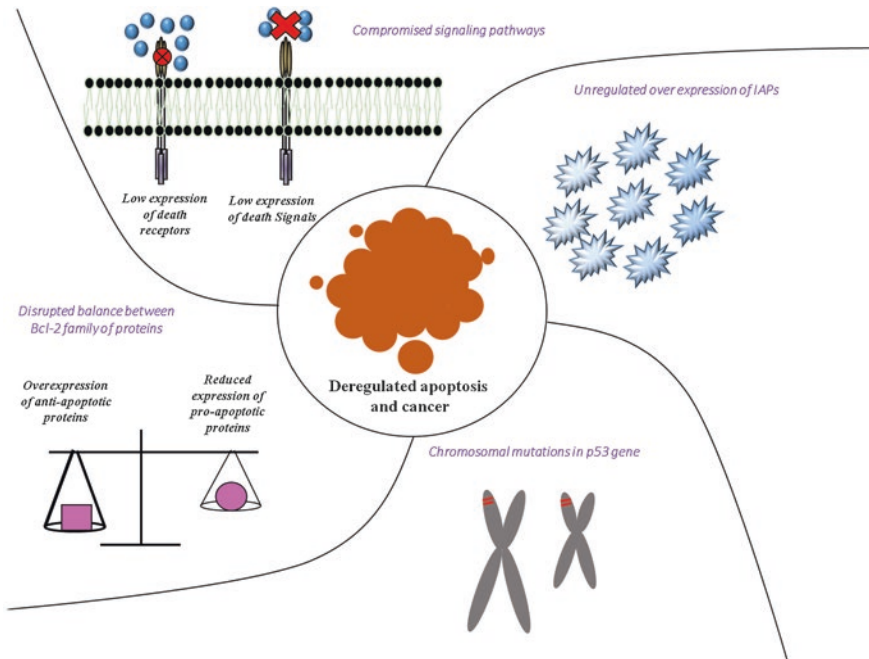
Fig. 5.3 Disrupted balance between Bcl-2 family of proteins: Many proteins have been reported to exert pro- or anti-apoptotic activity in the cell. It is not the absolute quantity but rather the ratio of these pro- and anti-apoptotic proteins that plays an important role in the regulation of cell death. Besides, over- and underexpression of certain genes have been found to contribute to carcinogenesis by reducing apoptosis in the cancer cells

5.3   Disrupted Balance of Pro-apoptotic and Anti-apoptotic Proteins

5.3.1   Role of Bcl-2 Family of Proteins in Apoptosis

This family of proteins is considered as a chief doorkeeper to the apoptotic response. Gene involving Bcl-2 family of proteins was first identified as one of the promoters of cell death whose functioning revolved around activation of pro-apoptotic proteins or inhibition of anti-apoptotic proteins. Anti- and pro-apoptotic members belonging to this group of structurally related proteins interact with each other. Among them, one of the subgroups include Bid, Bad, Bim, Bmf, Puma, and Noxa proteins that contain a single Bcl-2 homology 3 domain (BH3-only proteins) and have pro-apoptotic activity. Two other protein subcategories have multiple BH domains. The first subcategory, including Bcl-2-associated X protein (Bax), Bcl-2 homologous antagonist/killer (Bak), and Bcl-2 family apoptosis regulator (Bok), is pro-apoptotic; the other subcategory, including Bcl-2, Bcl-XL, and Mcl-1, is anti-apoptotic (Fig. 5.4). At least one BH domain is confined in each of the Bcl-2 family members and contributes to the functioning of the members (Strasser et al. 2011).
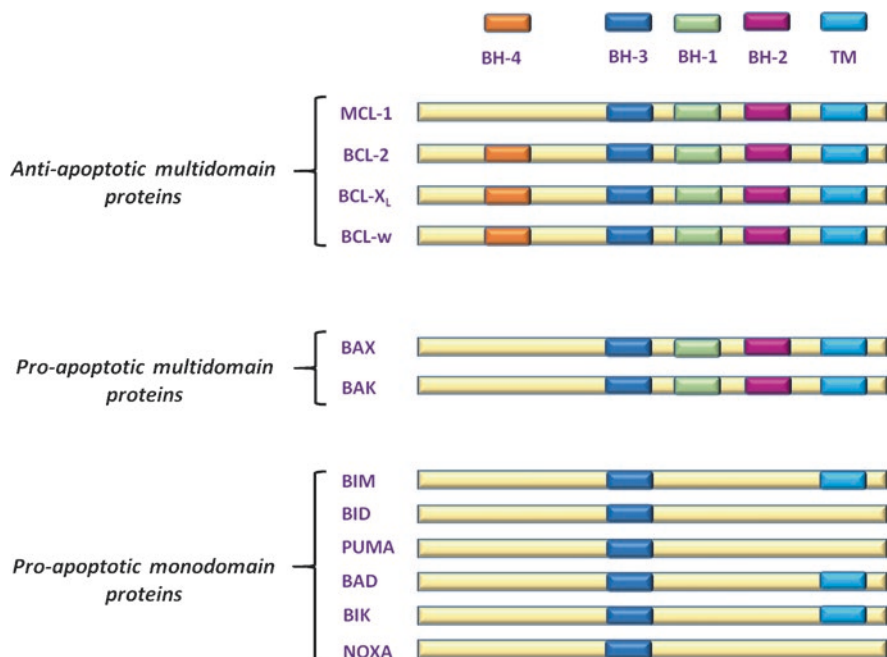
Fig. 5.4 Bcl-2 family: organization, functions, and characteristics. Schematic shows the important Bcl-2 family proteins in humans along with the main conserved structural motifs (BH domains), grouped according to function. TM, transmembrane domain. BH, Bcl-2 homology

The Bcl-2 family members can be classified into 3 functional groups: anti-apoptotic, pro-apoptotic *effectors*, and pro-apoptotic *activators*. According to a number of literature reports, pro-apoptotic activators, which comprise BH3 motifs, mostly function as important mediators in stress-related cellular responses like DNA damage (Certo et al. 2006). However, *effectors* group of Bcl-2 proteins initiates pore formation in the mitochondrial membrane after being activated by BH3 containing *activators* (Garcia-Saez 2012; Wei et al. 2001).

However, in preclinical models, it has been established that Bcl-2 binding to BH3-only *activators* disrupts the interactions between *activators* and pore-forming *effectors*, which in turn refrain the *effectors* from forming mitochondrial pores. The dynamic balance occurring between anti-apoptotic and pro-apoptotic members may involve combinations of under- or overexpression of one or more anti-apoptotic as well as pro-apoptotic proteins. This further helps to determine whether the cell initiates apoptosis or not (Strasser et al. 2011; Garcia-Saez 2012). For example, there are reports on Bcl-2 overexpression resulting survival of prostate tumor cells from apoptosis (Raffo et al. 1995). Moreover, in breast carcinoma and neuroblastoma cancer cells, overexpression of Bcl-2 is observed which leads to reduced TRAIL-induced apoptosis (Fulda et al. 2002). Similarly, various in vivo studies show that in chronic lymphocytic leukemia (CLL) cells, Bcl-2/Bax ratio increases with the decrease in pro-apoptotic Bax protein. Moreover, it is also seen that apoptosis

induced by drug application decreases the ratio and rate of drug induction is inversely proportional to the ratio (Goolsby et al. 2005).

5.3.2   Tumor Cells May Become Dependent on Bcl-2 for Survival

Recently, a phenomenon known as "oncogene addiction" has been identified. It is the dependence of a cancer cell on one signaling pathway or hyperactive gene for the cell's survival and growth. Similar oncogene addiction is also seen in various cancer cells whose survival become directly dependent on pathways involving Bcl-2 proteins (Certo et al. 2006). In case of these tumor cells, the pro-apoptotic *activators* get sequestered due to Bcl-2 binding. However, with a sufficient increase in the *activators* quantity, Bcl-2 sequestering by those *activators* can be invoked resulting apoptosis. Hence, this sensitivity toward Bcl-2 modulation can be used as a therapeutic tool for the treatment of Bcl-2-dependent cancers (Deng et al. 2007).

5.4   Expression Levels in Various Cancers

Expressions of functional Bcl-2 (increased ratio of anti-apoptotic to pro-apoptotic proteins) alone can regulate the extent of developing cancer. Following are the different cancer types and its association with aberrant expression of Bcl-2 blood *cancer/leukemia.*

CLL patients have always shown a noticeable Bcl-2 gene rearrangement with upregulated expression (Hanada et al. 1993). Increased expression is also found frequently in **a**cute **m**yeloid **l**eukemia (AML) (Wei et al. 2001) and in almost all patients with **a**cute **l**ymphocytic **l**eukemia (ALL) (Gala et al. 1994).

5.4.1   Non-Hodgkin lymphoma

In follicular lymphoma, most of the patients shows a t(14;18) chromosomal translocation in abnormal cells (Tsujimoto et al. 1984), which is believed to cause overexpression of Bcl-2 protein (Chen-Levy et al. 1989). This is true with diffused large B-cell lymphoma patients, in whom relatively high Bcl-2 expression is found (Hermine et al. 1996).

5.4.2   Cancerous Solid Tumors

Involvement of Bcl-2 in non-hematologic cancers like small cell carcinoma, breast carcinoma, and prostate and lung cancers has been observed where its unregulated expressions lead to aforementioned malignancies (Karnak and Xu 2010; Hellemans et al. 1995; Jiang et al. 1995; Anagnostou et al. 2010). In case of lung cancer, overexpression in >85% of patients has been reported (Jiang et al. 1995). Moreover, in

Table 5.1 Clinical evidences in cancer

Malignancy	Bcl-2 expression levels
Chronic lymphocytic leukemia (CLL)	Relatively high levels are observed in more than 90% of CLL cases compared to normal peripheral blood lymphocytes (Hanada et al. 1993)
Acute myeloid leukemia (AML)	Reduced response to chemotherapy at high level of expression (Campos et al. 1993)
Acute lymphocytic leukemia (ALL)	High levels are found in nearly all patients with ALL (Gala et al. 1994).
Follicular lymphoma (FL)	Chromosomal translocation resulting in overexpression in nearly 90% of follicular B-cell lymphomas (Tsujimoto et al. 1984)
Diffuse large B-cell lymphoma (DLBCL)	Chromosomal translocations-induced Bcl-2 overexpression is also seen in approximately 20% of DLBCL (Huang et al. 2002). Overall survival rate within a particular subgroup of DLBCL is inversely proportional to Bcl-2 expression (Iqbal et al. 2006).
Cancerous solid tumors	Untamed Bcl-2 levels have been observed in various tumors like breast, prostate, and lung cancers (Karnak and Xu 2010; Hellemans et al. 1995; Jiang et al. 1995; Anagnostou et al. 2010).

neuroblastoma, colorectal, ovarian, and urinary bladder cancers, substantial levels of Bcl-2 expression have been observed (Henriksen et al. 1995; Lamers et al. 2012; Swellam et al. 2004; Zhao et al. 2005; Pena et al. 1999) (Table 5.1).

5.5   The p53 Protein

Tumor protein 53 (or TP 53) is a tumor-suppressor protein encoded by the *TP53* gene, due to its specific molecular weight, that is, 53 kDa. The association of p53 with cancer surfaced in the late 1990s, which showed that gain of its oncogenic function was the direct outcome of its mutation (Levine et al. 1991). p53 is widely known as the "guardian of the genome" due to its significant role in a large number of cellular processes like chromosomal segregation, cell cycle regulation, recombination of genetic material, gene amplification, as well as apoptosis (Oren and Rotter 1999; Lane 1992). More than 50% of different types of human cancers are associated with malfunctioning of p53 gene (Levine et al. 1991). Recently, abnormal expression of p53 has also been reported in melanoma cells where its reduced activity leads to melanoma cells proliferation (Avery-Kiejda et al. 2011) Similarly, one of the research groups observed decreased survival, reduced apoptosis, and enhanced level of proliferative markers in a mouse model expressing truncated version of p53(Δ122p53) (Slatter et al. 2011). Moreover, p53 knockdown also decreases the

growth of cellular colonies in malignant tumor cells, which is due to apoptotic induction (Vikhanskaya et al. 2007).

5.6   Inhibitors of Apoptosis Proteins (IAPs)

Regulation of apoptosis involves a group of structurally related and functionally analogous group of proteins called the IAPs. The family is characterized by having one or two Baculovirus IAP repeat (BIR) protein domain (LaCasse et al. 2008). Since then, eight IAPs have been discovered, namely, Apollon (BRUCE, BIRC6), c-IAP1 (BIRC2), c-IAP2 (BIRC3), IAP-like protein 2 (BIRC8), Livin/ML-IAP (BIRC7), NAIP (BIRC1), survivin (BIRC5), and X-linked IAP (XIAP, BIRC4) (Vucic and Fairbrother 2007). Generally, they are involved in inhibition of caspase activity by either degrading the active forms of caspases or holistically interacting with them to prevent substrate-caspase binding (Wei et al. 2008).

It has been reported that in many cancers, there is a versatile expression of IAP proteins. For example, unusual levels of IAP were identified in the pancreatic cancer cells due to its link with chemoresistance. A study has further reported that there is a significant increase in the levels of cIAP-2 in many cancers (Lopes et al. 2007). According to recent reports, in cancers such as melanoma and lymphoma, significantly high levels of Livin/ML-IAP (BIRC7) has been reported (Vucic et al. 2000; Ashhab et al. 2001).While in gliomas, Apollon (BRUCE, BIRC6) upregulation exhibited chemoresistance for drugs cisplatin and camptothecin (Chen et al. 1999). Overexpression of survivin in various cancers has also been described. Together with XIAP, overexpression of survivin has been observed in non-small-cell lung carcinomas (NSCLCs). According to their study, tumors that upregulate both the proteins are capable of chemoresistance in all the apoptosis-inducing conditions (Krepela et al. 2009).

5.7   Potential Treatment Strategies Against Cancer

Role of dysregulated apoptosis pathways not only is limited to initiation of malignancy and cancer development but also leads to chemo- and radiation-resistant tumor cells. Initial responses of the cancer cells toward different therapies subside gradually as the cells start resisting apoptosis by increasing anti-apoptotic protein expressions which in turn block the pro-apoptotic pathways. Therefore, modern cancer research and drug development predominantly involve in finding the fundamental mechanisms governing apoptosis which may help in constructing therapeutic strategies to combat with cancer resistance and enhance patient survival.

5.7.1 Bcl-2 Antagonists

Bcl-2 family of proteins can be therapeutically targeted by developing Bcl-2 antagonists which may inhibit their activities as well as silence the upregulated anti-apoptotic genes or proteins. Significant role of Bcl-2 proteins in disrupting apoptosis has led to several drug-based advances in twenty-first century, which focus mainly on developing molecules that antagonize Bcl-2 family of proteins and quantitatively reduce their levels in cells. For example, a drug has been developed on the basis of an antisense nucleotide to *bcl-2* and has been successfully tested in a wide range of human cancers (Frankel 2003). Moreover, Bcl-2 antagonists have been designed by a number of research groups to primarily mimic BH3-only peptides (Baell and Huang 2002; Kutzki et al. 2002; Becattini et al. 2004; Qian et al. 2004). These agents are useful in treating cancers such as follicular lymphoma in which elevated levels of Bcl-2 occur.

5.7.2 SMAC (Second Mitochondria-Derived Activator of Caspase)/Diablo Mimetics

Development of small molecule and peptide mimetics that mimic SMAC can be another novel approach as it is a pro-apoptotic mitochondrial protein that is also an endogenous inhibitor of the IAP family of cellular proteins.

IAPs represent the last line of defense for the cancer cells against apoptosis. Clinically, IAPs have been proven to be a significant factor in cancer cell survival, development, and poor prognosis. Their association with tumor resistance to therapies is considered as one of the important therapeutic targets to selectively induce apoptosis in tumor cells.

The main objective behind designing SMAC mimetics (also called IAP antagonists) is to downregulate cellular IAPs that ultimately induce cancer cell death.

One of the SMAC mimetics, *TL32711,* in its clinical studies on patient-derived tumor xenograft models has successfully led to tumor regression.

Moreover, it also helps in preserving cancer cell sensitivity toward stimulation generated by pro-apoptotic elements like TNF-α or TRAIL, as shown in an in vitro human-derived cancer cell lines.

Collectively, these mimetics has been a great deal in cancer research and therapeutics with great potential to overcome the limitations of current anticancer therapies.

5.7.3 MDM2-p53 Complex

Novel agents that bind MDM2 (mouse double minute 2 homolog) have been developed that displace p53 from the complex, thereby activating the p53 pathway. p53 activation subsequently leads to cell cycle arrest and apoptosis (Vassilev et al. 2004).

Development of such agents as drugs would have the advantage of specifically targeting tumors overexpressing MDM2, leaving the normal cells aside.

5.7.4 Death Receptor Ligands (TNF and TRAIL)

Tumor necrosis factor (TNF) is a well-known anticancer agent in animal models, and has been found to cause death of tumor cells. Interestingly, it acts on the surrounding cells that are associated with feeding the tumor (Stoelcker et al. 2000).

Another important molecule, TRAIL (LeBlanc and Ashkenazi 2003), acts directly on cancer cells and causes apoptosis. It has been well established that TRAIL knockout mice works hand in hand with natural killer (NK) cells in order to keep metastasis from happening (Takeda et al. 2001; Cretney et al. 2002). Moreover, induction of cell death has also been observed in tumor cells from various cancer patients due to the activation of TRAIL-producing macrophages which further release biological molecules that consequently result in upregulation of particular markers on tumor cells (Herbeuval et al. 2003).

5.7.5 Monoclonal Abs (Rituximab) and Apoptosis in Cancer

One of the most potent agents for treatment of lymphocytic cancer such as non-Hodgkin's lymphoma is a monoclonal antibody (Rituximab) against the antigen B220 present in B-cells. This monoclonal antibody mainly acts by inducing apoptosis that ultimately leads to cell death (Cartron et al. 2004).

5.8 Conclusion

Cancer Is a Failure of Apoptosis
Apoptosis or programmed cell death is one of the ways through which the cell cycle is maintained and kept under check. This basic defense mechanism has always been the main reason why cancer cells do not thrive and lead to abnormal cell growth or cancer. For example, cells from the epidermal layer of the skin when exposed to harmful ultraviolet radiations in this case as a part of defense mechanism, programmed cell death is normally activated.

This helps in eliminating those injured cells that otherwise would survive and develop into cancerous growths.

In metastasis or malignant cancer, in order be alive, cells always travel from one organ to the other through the blood system.

This is usually prevented by apoptosis as cells typically "self-destroy" when they lose contact with neighboring cells or the extracellular matrix (Hanahan and Weinberg 2000).

One of the hallmarks of cancer is to escape apoptosis (Hanahan and Weinberg 2011). Cancer cells display many characteristics; for example, they interfere into

cell cycle checkpoints and withstand exposure to cytotoxic agents (Letai 2008) that subsequently lead to their survival. Since apoptosis is an important impediment toward developing cancer, avoiding apoptosis is integral to tumor development and resistance to therapy (Cory et al. 2003; Plati et al. 2011). Cancer cells are capable of escaping apoptosis and continuously dividing. One of the important reasons for this behavior is loss of tumor-suppressor p53.

p53 knockout cells are incapable of sensing DNA damage that drives apoptosis (Hanahan and Weinberg 2000). Anti-apoptotic Bcl-2 family members and IAPs also promote cell proliferation when upregulated (Vo and Letai 2010). Although there has been a huge advancement in understanding the apoptotic pathway and targeting it for therapeutic intervention, the challenges in this area are still manifold. Complex machineries of cancer cells enable them to develop resistance to apoptosis by acquiring new mutations which nullify the drug-induced targeted therapies. For example, apoptosis is initiated in cancer cells when drug inhibits the activity of Bcl-2 family proteins. However, a new mutation generated in the cancer cells may upregulate the caspase inhibitors, and the drug will not be effective anymore (Wong 2011). Therefore, a comprehensive understanding of the apoptotic pathway along with the cross talks among different pro- and anti-apoptotic proteins might open new avenues in devising new strategies to effectively combat cancer.

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