The Importance of Apoptosis in Cancer Development and Treatment

İrem Nur GÖKBAYRAK ATAY,1 Ayhan Hilal GEZER,2 Elmas KASAP3

1Department of Neuroscience, Dokuz Eylül University Faculty of Health Sciences Institute, İzmir-Turkey
2Department of Medical Biology, Celal Bayar University Faculty of Medicine, Manisa-Turkey
3Department of Internal Medicine and Gastroenterology, Celal Bayar University Faculty of Medicine, Manisa-Turkey

SUMMARY
Cancer is one of the most important causes of death in our era. Multifactorial causes are involved in the formation of cancer. The reduction of apoptosis is one of these reasons. Failure to activate apoptosis pathways can lead to resistance to the current treatment approaches of cancers. A better understanding of the molecular events that regulate apoptosis in cancers and cancer therapy form the basis of a more rational approach to the development of molecular targeted therapies in the fight against cancer.

Keywords: Apoptosis; apoptosis pathways; cancer; cancer development; cancer treatment.

Introduction
Apoptosis is the programmed death of the cells, which are not needed and whose functions are impaired without harming the environment, as a requirement of intercellular relations in developed organisms. Starting from the embryo period, there is an apoptotic mechanism and programmed cell death throughout life. Some cells live for years, while some live only a few hours. Continuity in many tissues such as skin, gastrointestinal system, and immune system is dependent on apoptosis and cell renewal.[1]

Apoptosis is one of the most acceptable mechanisms for anticancer activity in cancer cells. The regulatory mechanisms of apoptosis are very complex. Reactive oxygen species, caspase activation, tumor necrosis factor (TNF), protein kinase, and mitochondrial pathway form the basis of apoptosis.[2]

What Is Cancer?
Cancer has been a common problem in humans and animals throughout the known history. The earliest known records about cancer date back to 3000 BC. The word cancer is derived from the words “canker” or “carcinos,” which means crab in Latin. The term tumor was first used by Hippocrates in the 3rd century BC because it compared the swollen veins around the tumor to the legs of a crab, and the term “oncos,” which means swelling, was used.[3]

Cancer is a complex disease caused by uncontrolled division and proliferation of cells and under the influence of genetic and environmental conditions. Cancer is also a personal disease, although there are more than 100 known types of cancer and standard approaches have been developed for certain types of cancers. Cancer is the uncontrolled division, proliferation, and accumulation of cells in an organism. It can affect a single organ as well as spread to distant organs and show its effect.[3] Cancer is a disease associated with a decrease in apoptosis.[4]

What Is Apoptosis?
Apoptosis is a word formed by the combination of ancient Greek apo (separate) and ptosis (fall) and used by
Homer to describe leaf fall in autumn. For this reason, it is the type of cell death that occurs when some cells dry up like autumn leaves and leave the body and make room for the cells coming from behind. It was named “apoptosis” with the suggestion of the classical Greek historian James Cormack.[5]

Apoptosis; it is programmed cell death, which is an important cell growth control component that ensures the elimination of cells that are not needed, whose functions are impaired, that have completed their biological task or are damaged after exposure to a certain stimulus.[6]

There are two pathways in apoptosis: Extrinsic pathway and intrinsic pathway.[7,8]

**Extrinsic Pathway (Receptor Pathway)**

This pathway is stimulated by the tumor necrosis factor-alpha (TNF-alpha) family, which binds to the CD95 ligand (Fas ligand=CD95L) by extracellular signals. This binding causes the formation of death-inducing signal complex (DISC) by causing conformational changes independent of ATP at the receptor. With the CD95 adapter molecule Fas receptor-associated measurement unit (FADD), FADD also combines with procaspase-8 and procaspase-10 to form the DISC complex. Separation of small and large subunits is not required for activation. The next step is activation of procaspase-8, causing proteolysis from caspase-3 to separate from the small subunit, thereby activating the enzyme. This pathway combines with the mitochondrial pathway through the caspase-3 activation, thereby strengthening the apoptosis signal. Cellular FLICE-inhibiting proteins (cFLIP, cFLIPL, cFLIPs) can combine with DISC to inhibit the activation of caspase-8 and 10, preventing apoptosis.[7,9]

**Intrinsic Pathway (Mitochondrial Pathway)**

Unlike this pathway death receptor pathway, it can be induced by both extracellular signals (growth factor or hormone deficiency, ultraviolet beam, and various cytokines) and intracellular signals that cause DNA damage. While the intracellular signal stimulates the proapoptotic members of the Bcl-2 family, it migrates to the mitochondria, while the proapoptotic members settle on the outer mitochondrial membrane and form pores (mitochondrial passages) here. These pores allow cytochrome-c separation from mitochondria. Cytochrome c; Combining with apaf-1, ATP, dATP, and procaspase-9, the apoptosome activates caspase-3. Although their beginnings are different, they both result in caspase-3 activation in both pathways. Furthermore, apoptosis-inducing factor (AIF) from mitochondria is released in molecules such as G, Smac/DIABLO, and Omi/HtrA2. Endonuclease G and AIF, while inducing DNA fragmentation, Smac/DIABLO, and Omi/HtrA2 neutralize apoptosis-inhibiting protein (inhibitor of apoptosis proteins [IAPs]). Molecules such as Smac/DIABLO and Omi/HtrA2 are located in the intermembrane region of the mitochondria, such as cytochrome c. Smac/DIABLO and Omi/HtrA2 have many similar aspects. However, while Smac/DIABLO is not found in the heart and detected in the brain, on the other hand, there is a widespread distribution of Omi/HtrA2.[7,9]

**Apoptosis and Cancer Relationship**

In cancer, an important disease associated with apoptosis, the balance between cell proliferation and cell death is disrupted, and cells are unable to receive death signals to cause apoptosis. This can cause problems at any step of the apoptotic pathways. Better understanding of the molecular events regulating apoptosis mechanisms enables the creation or development of new treatment options for the activation or inhibition of the target molecules in these pathways.[10]

The irregularity of apoptotic pathways not only promotes tumor formation but can also make the cancer cell resistant to treatment. Therefore, irregularity of apoptosis is an important cancer symptom.[11]

As with B cell lymphoma, decreased apoptosis in some tumors can cause tumor development. In general, an increase in apoptosis is observed due to the increase in proliferation in tumor tissue.[12]

Disorders in programmed cell death (apoptosis) mechanisms play important roles in tumorogenesis. Apoptosis allows neoplastic cells to survive over their intended lifespan, reduces the need for exogenous survival factors, and provides protection from oxidative stress and hypoxia as the tumor mass expands.[13]

It has been found that anti-apoptotic Bcl-2 subgroup is induced in many types of cancer. And it has been determined that the levels of inhibitors are induced in tumor cell lines. Tumor by inducing the molecule level can cause depopulation or apoptosis. For example, IL-3 induces the Bcl-xL level to improve survival of the myeloid sequence and interleukin IL-5 and IL-15 protect eosinophils and mast cells from apoptosis by inducing the Bcl-xL level.[14]
In chronic lymphocytic leukemia, IL-4 levels increase, excessive production of Bcl-2 occurs, and the life span of malignant B cells is prolonged. Two anti-apoptotic cytokines IL-4 and IL-10 are released in thyroid carcinoma cells and contribute to cancer development. There are kinds of molecular mechanisms that tumor cells use to suppress apoptosis. Tumor cells can become resistant to apoptosis by the expression of anti-apoptotic proteins such as Bcl-2 or by down-regulation or mutation of pro-apoptotic proteins such as BAX. Some forms of human B cell lymphoma have Bcl-2 overexpression. This example represents the first and strongest evidence that the absence of apoptosis contributes to cancer.

**p53 and Cancer**

The first gene therapy product used in cancer treatment is Gendicine, which is specifically designed to express p53 (rAd-p53). In a study conducted on patients with oral cancer, it has been shown that administration of rAd-p53 infusion together with chemotherapy significantly increases the survival rate in patients. Apart from that, it has been stated that it is used effectively in hepatocellular carcinoma, tongue cancer, and some other types of cancer.

Regulation of apoptosis in humans is a process that starts with p53 and continues up to caspases. Cell life is prolonged when p53, which works as a tumor suppressor gene, is mutated or absent. Cell damage caused by genotoxic events activates a transcription regulator gene, p53. After the p53 protein product binds directly to DNA and recognizes the damage, it either induces cell cycle arrest in G1, gaining time for repair, or directs apoptosis if the damage is greater. In addition, p53 is thought to regulate the ratios of Bax/Bax, Bax/Bcl-2, and Bcl-2/Bcl-2 groups.

**Caspases and Cancer**

Although the mechanism of apoptosis is not fully explained, the most important event associated with apoptosis is the activation of caspases.

Initiator caspases transmit death signals initiated by apoptotic stimulation to effector caspases. Effector caspases, on the other hand, break down related proteins and cause the formation of apoptotic cell morphology. Defects in the caspase can contribute to autoimmune diseases, cancer, and the formation of some neurological disorders.

**IAPs and Cancer**

IAP (inhibitors of apoptosis), a family of caspase inhibitors, selectively inhibit caspases. Thus, they stop the apoptotic mechanism. These inhibitors are expressed by many malignant cells. IAPs can also stop apoptosis by affecting the cell cycle.

IAPs are a group of structurally and functionally similar proteins that regulate apoptosis, cytokinesis, and signal transduction. They are characterized by the presence of a baculovirus IAP repeat (BIR) protein domain.

Irregular IAP expression has been reported in many cancers. For example, Lopes et al., showed that the abnormal expression of the IAP family in pancreatic cancer cells and this abnormal expression is also responsible for resistance to chemotherapy. Among the IAPs tested, the study concluded that drug resistance was the most significant correlation with cIAP-2 expression in pancreatic cells.

Survivin, another IAP, has been reported to be overexpressed in various cancers. Small et al., transgenic mice overexpressing Survivin in hematopoietic cells are high in terms of hematological malignancy risk and observed that hematopoietic cells designed to overexpress Survivin are less susceptible to apoptosis.

Survivin has been found to be overexpressed in non-small cell lung carcinomas (NSCLC) in conjunction with XIAP. It has been concluded that Survivin overexpression in most NSCLCs is accompanied by excess or upregulated expression of XIAP.

**Carcinogenesis by Intrinsic Apoptosis Pathway Mutations**

The intrinsic apoptosis pathway is one of the most important pathways for apoptosis induction. Therefore, disrupting this pathway is an effective way of preventing apoptosis. Bcl-2 family proteins are central regulators of the intrinsic pathway, either suppressing or promoting changes in mitochondrial membrane permeability required for the release of cyt-c and other apoptogenic proteins.

Antia apoptotic proteins block the death signal by partially antagonizing the effects of Bax/Bak through known mechanisms. Furthermore, antia apoptotic proteins prevent Bax/Bak activation by sequestrating inhibiting BH3-only proteins “activator” and/or directly inhibiting Bax/Bak activation.
Antiapoptotic proteins (e.g., Bcl-2, Bcl-xL, Bcl-W, Mcl-1, and Bfl-1/A1, which exhibit sequence homology in all BH1-BH4 domains) increase cell survival, while proapoptotic proteins are receptor, endoplasmic reticulum (ER), or mitochondria. ER mediates stress-induced apoptosis. The next group contains proteins that contain the multi-domain or BH3-only. The first consists of Bax and Bak, which are necessary for apoptosis.[31]

The central role played by Bax/Bak in apoptosis is supported by studies that BH3-only proteins do not induce apoptosis in cells with Bax/Bak deficiency.[32,33]

Carcinogenesis by Extrinsic Apoptosis Pathway Mutations

It is activated in vivo by TNF family ligands that bind DD-containing receptors, which means that DED-containing caspases lead to its activation. "Death ligands" are expressed on CTLs, NK cells, and other immune-related cells (activated monocytes/macrophages and nitrite cells) and are used as weapons for destroying transformed cells.[34]

Another molecule, c-FLIP, which regulates caspase-8 activation, is c-FLIP (L), which are long IL-1β converting enzyme inhibitor proteins similar to cellular FADD. Another molecule, c-FLIP, which regulates caspase-8 activation, is c-FLIP (L), which are long interleukin-1 beta converting enzyme inhibitor proteins similar to cellular FADD.[35,36]

Melanoma, hepatocellular carcinoma, non-small cell lung carcinoma[37] and endometrial,[38] colon,[39] and prostate cancer,[40] including increased expression of c-FLIP has been detected in many human malignancies. Its overexpression is associated with cancer progression and/or poor prognosis in BL, HCC, and ovarian, endometrial, colon, and prostate cancer.[35,36,39]

The high expression of C-FLIP blocks caspase-8 and makes cells resistant to cell receptor-mediated apoptosis.[35] Overexpression of C-FLIP has been reported to bind proteins involved in these signaling pathways, including TRAF1, TRAF2, RIP, and RAF1, thereby promoting the activation of NF-κB and ERK as downstream molecules.[36]

Mice-based studies on the use of neutralizing antibodies and Fc-fusion proteins as well as genetic changes in genes encoding death ligands or their receptors, found that death ligands and the genes encoding them play important roles in tumor suppression by cellular immune mechanisms. aFas ligand (FasL) is important for CTL-mediated killing of some tumor targets, and TRAIL (Apo2 ligand) is critical for NK-mediated tumor suppression. Some tumor cells resist the death receptor pathway's response to FasL produced by T cells to avoid immune damage. Many tumor cells show intrinsic resistance to TRAIL. This directly sequesters Fas of ligand or expression Fas ligand on the surface of tumor cells. This is downregulation of the Fas receptor, a dysfunctional Fas receptor, and it creates the secretion of high levels of Fas receptor.[41] Some tumor cells may undertake a Fas ligand-mediated “counterattack” causing apoptotic depletion of active tumor-infiltrating lymphocytes.[42]

Apoptosis and Cancer Treatment

Cancer-related defects in apoptosis play a role in resistance to treatment with traditional treatments such as chemotherapy and radiotherapy, increasing the cell death threshold, thus requiring higher doses of tumoricidal agents.[43]

Apoptosis and Cancer Theratment targets apoptosis via receptor-chondrial-mediated. Many drugs are used in cancer treatment today to kill cells. Disruption of mitochondrial membrane potential, cytochrome c release, and activation of different caspases have been identified following treatment of cells with different chemotherapeutic agents (Table 1).[44,45]

Tumor selective expression of proapoptotic Bax by adenoviral gene transfer leads to selective toxicity in tumor cells.[46]

Bortezomib has a sensitive effect on apoptosis induced by NF-κB suppression by downregulated Bcl-2.[47] On the other hand, it has been reported that BH3 proteins Bik and Bim levels, which are an important mediator of antitumor activity, increase in various cell lines through bortezomib.[48] Heat shock proteins are also pharmacological targets. Geldanamycin, an Hsp 90 inhibitor, shows a clear antitumor effect.[49] The drug known as eurycoma has proven excellent antiproliferative and anticancer effectiveness against a variety of human cancers in most in vitro and in vivo studies. Eurycoma mechanism of action; it is the induction of apoptosis and down-regulation of expression by up-regulation of p53 (tumor suppressor protein) and proapoptotic protein (Bax) expression and down-regulation of expression.[50]
<table>
<thead>
<tr>
<th>Treatment strategy</th>
<th>Remarks</th>
</tr>
</thead>
</table>
| Targeting the Bcl-2 family of proteins                  | **Agents that target the Bcl-2 family proteins**

Oblimersen sodium
- Reported to show chemosensitizing effects in combined treatment with conventional anticancer drugs in patients with chronic myeloid leukemia and an improvement in survival in these patients

Small molecule inhibitors of the Bcl-2 family of proteins
- Molecules reported to affect gene or protein expression include sodium butyrate, depsipeptide, fenretinide, and flavipirodo. Molecules reported to act on the proteins themselves include gossypol, ABT-737, ABT-263, GX15-070, and HA14-1 BH3 mimetics

ABT-737 reported to inhibit anti-apoptotic proteins such as Bcl-2, Bcl-xL, and Bcl-W and to exhibit cytotoxicity in lymphoma, small cell lung carcinoma cell line, and primary patient-derived cells

ATF4, ATF3, and NOXA reported to bind to and inhibit Mcl-1

**Silencing the Bcl family anti-apoptotic proteins/genes**

Bcl-2 specific siRNA reported 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

Silencing Bmi-1 in MCF breast cancer cells reported to downregulate the expression of pAkt and Bcl-2 and to increase sensitivity of these cells to doxorubicin with an increase in apoptotic cells *in vitro* and *in vivo*

**Targeting p53**

**p53-based gene therapy**
- First report on the use of a wild-type p53 gene containing retroviral vector injected into tumor cells of non-small cell lung carcinoma derived from patients. The use of p53-based gene therapy was reported to be feasible
- Introduction of wild type p53 gene reported to sensitize tumor cells of head and neck, colorectal and prostate cancers, and glioma to ionizing radiation

Genetically engineered oncolytic adenovirus, ONYX-015 reported to selectively replicate in and lyse tumor cells deficient in p53

Small molecules
- Phikan083 reported to bind to and restore mutant p53
- CP-31398 reported to intercalate with DNA and alter and destabilize the DNA-p53 core domain complex, resulting in the restoration of unstable p53 mutants

Other agents
- Nutlins reported to inhibit the MSM2- p53 interaction, stabilize p53, and selectively induce senescence in cancer cells
- MI-219 reported to disrupt the MDM2-p53 interaction, resulting in inhibition of cell proliferation, selective apoptosis in tumor cells, and complete tumor growth inhibition
- Tenovins reported to decrease tumor growth *in vivo*

**p53-based drug therapy**

Patients with advanced-stage cancer given vaccine containing a recombinant replication-defective adenoviral vector with human wild-type p53 reported to have stable disease

Clinical and p53-specific T cell responses observed in patients given p53 peptide-pulsed dendritic cells in a Phase I clinical trial
<table>
<thead>
<tr>
<th>Treatment strategy</th>
<th>Remarks</th>
</tr>
</thead>
</table>
| **Targeting IAPs** | Antisense approach  
An improved *in vivo* tumor control by radiotherapy  
Concurrent use of antisense oligonucleotides and chemotherapy reported to exhibit enhanced chemotherapeutic activity in lung cancer cells *in vitro* and *in vivo*  
siRNA approach  
siRNA targeting of XIAP reported to increase radiation sensitivity of human cancer cells independent of TP53 status  
Targeting XIAP or Survivin by siRNAs sensitized hepatoma cells to death receptor, chemotherapeutic agent-induced cell death  
Antisense approach  
Transfection of antisense Survivin into YUSAC-2 and LOX malignant melanoma cells reported to result in spontaneous apoptosis  
Reported to induce apoptosis and sensitize head and neck squamous cell carcinoma cells to chemotherapy  
Reported to inhibit growth and proliferation of medullary thyroid carcinoma cells  
siRNA approach  
Reported to downregulate Survivin and diminish radioresistance in pancreatic cancer cells  
Reported to inhibit proliferation and induce apoptosis in SPCA1 and SH77 human lung adenocarcinoma cells  
Reported to suppress Survivin expression, inhibit cell proliferation, and enhance apoptosis in SKOV3/DDP ovarian cancer cells  
Reported to enhance the radiosensitivity of human non-small cell lung cancer cells  
Small molecules antagonists  
Cyclin-dependent kinase inhibitors and Hsp90 inhibitors and gene therapy attempted in targeting Survivin in cancer therapy  
Cyclopeptidic Smac mimetics 2 and 3 report to bind to XIAP and cIAP-1/2 and restore the activities of caspases-9 and 3/-7 inhibited by XIAP  
SM-164 reported to enhance TRAIL activity by concurrently targeting XIAP and cIAP1 |
| **Targeting Survivin** |  |
| **Other IAP antagonists** |  |
| **Targeting caspases** | Apoptin reported to selectively induce apoptosis in malignant but not normal cells  
Small molecules caspase activators reported to lower the activation threshold of caspase or activate caspase, contributing to an increased drug sensitivity of cancer cells  
Human caspase-3 gene therapy used in addition to etoposide treatment in an AH130 liver tumor model reported to induce extensive apoptosis and reduce tumor volume  
Gene transfer of constitutively active caspase-3 into HuH7 human hepatoma cells reported to selectively induce apoptosis  
A recombinant adenovirus carrying immunocaspase 3 reported to exert anticancer effect in hepatocellular carcinoma *in vitro* and *in vivo* |
Conclusion

Demonstrating the relationship between the cell apoptosis system and its elements with cancer may allow the causes of cancer to be determined and resolved in a case and effect relationship. In addition, it may open new horizons in cancer treatment using agents associated with the apoptotic system against cancer. Thus, effective cancer treatment can be demonstrated.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Authors declare no conflict of interest.

Financial Support: No financial support has been used for this study.

References


