REVIEW ARTICLE

Apoptosis in cancer therapy
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Abstract
In third world countries like India, due to adverse oral habits like tobacco chewing oral cancers are one of the main health problems. Understanding and identifying markers that help us identify cancer early before the consequences become clinically and histologically detectable will greatly enhance the prognosis. Apoptosis is one such early diagnostic marker for cancer and is programed, active process that dismantles the cellular components without evoking an inflammatory response. It is known to remove all mutated and potentially malignant cells; hence, control of apoptosis is considered to play a key role in carcinogenesis. Through adequate cancer therapies, apoptotic programs can be modified to produce potential drug targets. Gene therapy in combination with surgery, radiotherapy, and chemotherapy has been an attractive method of cancer treatment. In the coming years, new therapies that are nontoxic than present treatment regimens will pave a new path in cancer research. The aim of this review is to understand the part of apoptosis in cancer treatment.

Keywords:
Apoptosis, Bcl-2, chemotherapy, caspases, endothelial growth factor receptor, p53, radiotherapy, tumor necrosis factor, tumor necrosis factor-related apoptosis-inducing ligand

Introduction
In third world countries like India, oral cancers are a huge health concern, mainly associated with tobacco chewing habit. In males, it is the most common cancer, and in females, it is the third most common. The majority of the oral cancers are epithelial in origin, known as squamous cell carcinomas. Early diagnosis is very important, as it improves the prognosis, the cure rate, with minimal deformity.

Identifying and understanding apoptosis has become important besides its prognostic significance. Although apoptosis and necrosis are two cell death forms, apoptosis is described as a planned active, independent program that causes cellular breakdown without evoking an inflammatory response. Normally, apoptosis is an equilibrium between pro-apoptotic genes and anti-apoptotic genes. When this balance tips off, it results in various ailments. Hence, the study of apoptosis is relevant in many aspects of tumor biology which includes tumorigenesis, tumor homeostasis, angiogenesis, metastasis, and also in clinical treatment.

The word apoptosis is derived from the Greek word, which refers to the petals falling off from flowers or leaves from trees. Kerr, in 1972, first coined the term “apoptosis” to describe, “programed cell death.” It is a method of cell death that is involved in normal cellular development and aging process, which is distinct from necrosis. It is an active, well regulated cellular process where individual cells are activated to endure self-extinction in a mode that neither injures the neighboring cells nor evoke any inflammatory reaction unlike necrosis.

Apoptosis in Cancer Therapy
Apoptosis that is involved in controlled elimination of mutated cells are involved in removal of potentially malignant cells. Hence, apoptosis regulation plays a key role in carcinogenesis, tumor growth, and metastasis. The controlled apoptotic death of cells reflects the accurate internal functioning of death machinery in an individual. Accumulation of genetic and epigenetic alterations leads to anomalous expression of proteins that regulate cell growth leading to cancer.

In many forms of cancer, including oral cancers, apoptosis is deregulated causing uncontrolled proliferation due to activation of certain oncogenes. This is supported by frequent impairment of apoptosis checkpoints. Many of the oncogene-driven cellular changes that bring about the malignant transformation, unregulated proliferation, invasion and metastasis actually prevent the transformed cells from undergoing apoptosis.
Acquiring additional defects in apoptosis pathways these cells survive, cause further invasion and distant metastasis.\(^6\)

**Apoptosis and Chemotherapy**

Cancer cells depend on abnormal apoptotic signaling pathways to remain immortal. Drugs that can repair the impaired apoptotic pathways have the potential for effective personalized treatment for cancers. Selective killing of tumor cells without any damage to the neighboring normal cells are our future therapeutic goals.\(^7\)

**Chemoprevention**

Chemoprevention is basically use of certain agents that can retard the progression of reverse or prevent the formation of cancer. Invariably, this minimizes the risk of malignant transformation and metastasis. An ideal chemopreventive agent should effectively block cancer formation in premalignant cells. It should also arrest the progression, invasion, and metastasis in malignant cells and destroy them.\(^9\)

Targeting apoptosis pathways by chemopreventive agents may be effective for cancer prevention.\(^10\) For example, in oral premalignant lesions, such as oral leukoplakia and actinic keratoses, natural and synthetic retinoids have been used effectively to prevent further progression. By controlling cell proliferation or differentiation, many chemopreventive agents (e.g., retinoids and antiestrogens) act against tumor progression.\(^8\)

**Chemotherapy**

Chemotherapy as an adjuvant therapeutic mode for cancer treatment was established more than 50 years ago. Drugs should be designed such that they decrease the tumor burden by inducing cytotoxic and/or cytostatic effects on cancer cells with no damage to normal cells.\(^8\) Chemotherapy along with radiotherapy has proven effective postsurgery in aggressive oral cancers.\(^9\)

Chemotherapeutic agents function by inducing death of cells by triggering the apoptotic pathway. However, the response to chemopreventive drugs is very different in each individual. Each one of us has a different genetic make-up and environmental conditions. Hence, the pattern of oncogene activation and tumor suppressor functions vary due to somatic cell genetic differences in each person, though the tumor has same tissue of origin.\(^10\)

Due to large extent of heterogeneity, sensitivity of apoptosis to certain drugs is lost, causing drug resistance.\(^11\)

For a tumor cell to grow and thrive, it has to overcome the antitumor immune response presented by the host. It may be deprived of nutrition and have to survive through extreme hypoxic state. It undergoes structural, metabolic changes as well as alteration in the genes expressed. These aberrations provide the cancer cells to acquire antiapoptotic properties, an intrinsic survival advantage, and drug resistance. This “double whammy” state allows further growth and proliferation of cancer cells and may hinder subsequent therapy.\(^9,12\)

Evading apoptosis by upregulation of antiapoptotic or downregulation of proapoptotic proteins is an important step in tumorigenesis and determines susceptibility to various chemotherapy and radiation modalities.

**p53**

One of the major challenges in current clinical trials is restoring the p53 signaling pathway. Apoptosis and tumor regression process is slowed down in the presence of mutant 53. An activation of immune response against cancer cells that express mutant forms of p53 are attempted. Elimination of mutant p53 is achieved by re-introduction of normal p53 to tumor lines and it is seen that this triggered apoptosis and caused tumor growth suppression. Disrupting the interactions of apoptotic proteins and mutant p53 can be done by activation of wild-type p53 function. Certain molecules that could prevent the degradation of p53 by MDM2 and activate the p53 function were identified. There are various factors, like agent, the drug dosage, tissue of origin, and the degree of mutation undergone, that contribute to apoptosis by p53 [Figure 1].\(^13\)

**Bcl-2**

Many human malignancies frequently show dysregulation of Bcl-2 family members that contributes to drug resistance. Increased drug resistance and radioresistance has been correlated to overexpression of Bcl-2 as well as other pro-survival Bcl-2 family members. Functional inhibition of members of Bcl-2 family using combination of drugs can definitely help in evading cancer. This will enhance and optimize the therapeutic effect of drugs.\(^14\)

**Caspases**

Systematic and controlled elimination of oncogenic cells by anti-oncogenic drugs require the activation of proteolytic cascade by the caspases. Activation of initiator caspases by anticancer drugs results in cleavage of a variety of cytosolic, cytoskeletal, nuclear, or other cellular proteins. These ordered proteolytic events amplify the apoptotic signal and bring systematic and orderly morphological variations in the cell undergoing apoptosis.\(^15\)

The key regulators of caspase activation are Bcl-2 protein members. After the proposed chemotherapy is completed, it is seen that mitochondrial cytochrome C is released before or concurrent with caspase activation. This is antagonized by BclXL overexpression.\(^13\)

**Tumor necrosis factor (TNF)**

TNF ligands are considered as mediators of cell death. It is known that as the tumor proliferates it requires more blood supply to
fulfill its nutritional needs. Destruction of tumor vasculature is an efficient way to combat cancer. It was seen that TNF when combined with melphalan, a chemotherapeutic agent killed cancer cells by this method.[16]

**TNF-related apoptosis-inducing ligand (TRAIL)**

TRAIL is a cytokine, a protein functioning as a ligand that induces apoptosis, by binding to certain death receptors.[17]

Targeting TRAIL in therapeutics is very effective as it particularly targets apoptosis only in oncogenic cells, sparing most normal cells. This paves a new path in cancer research as it promises biologically-targeted cancer chemotherapy.

Many conventional and novel agents can synergistically act with TRAIL to destroy oncogenic cells as many of these cells are against apoptosis caused by TRAIL. It will be an essential therapeutic strategy to destroy cancers with functional p53.[17]

**FLICE-inhibitory protein (FLIP)**

FLIP is an important regulator of death receptor signaling. FLIP blocks cell death triggered by TRAIL, by interfering with activation of caspase-8. Down-regulating FLIP can serve as a therapeutic target induction by TRAIL to sensitize tumor cells to apoptosis. A potential compound was discovered recently, known as CDDO, a synthetic oleanane triterpenoid that can reduce FLIP expression. This can effectively differentiate, prevent inflammation, and proliferation reducing the tumor growth in vivo. CDDO activates a pathway that can cause apoptosis resulting in FLIP degradation and caspase-8 cleavage and in combination with TNF or TRAIL, it has shown potent synergistic effect.[18]

**Targeted Cancer Therapies**

Targeted cancer therapies aim at blocking or interfering with the definite molecules that take part in cancer cell proliferation. They target only cancer cells sparing normal cells as these “molecular targets” focus only on genetic and epigenetic changes that are cancer specific.[19]

These therapies intervene with the division and proliferation of cancer cells causing cell death by involving in the apoptotic cell signaling pathways. By blocking the mutated regulatory signals, there will be variations in the cellular activities, such as cell growth and division, its movements and responses to external stimulus that includes tumor cell death. Other targeted individualized therapies kill the tumor cells by two ways: Direct method: By targeting specific tumor cells and inducing apoptosis, indirect method: By stimulating the immune system of the host to recognize and destroy cancer cells by directing toxic elements toward them.[10]

**Apoptosis and Radiotherapy**

Since the last century, ionizing radiation has been used for the treatment of human cancers effectively. Radiotherapy is usually advised postsurgery in aggressive cancers to reduce the cancer recurrence risk, to control the tumor growth and improves the prognosis (patient survival). The aim of radiotherapy is to kill tumor cells while preventing the deleterious effect of radiation to nearby normal tissue. Due to recent innovations in the field of computers, technology and engineering, sophisticated irradiation techniques, including intensity-modulated radiotherapy the outlook of treatment planning has changed.[20]
Ionizing radiation works efficiently, when properties of tumor DNA specific agent are combined with higher degree of spatial specificity. The treatment outcome varies depending on the histological type of various types. Tumors of germ cell or lymphoid origin are radiosensitive and are easily manageable than most solid oral cancers.[21]

Tumor control using radiotherapy depends largely on tumor cell intrinsic radiosensitivity. Combination therapy is advised for better treatment outcome (surgery, chemotherapy or immunotherapy) with direct and indirect action. Reports suggest that radiotherapy used before surgery (neoadjuvant therapy), shrinks the tumor, reducing its size. Postsurgery (adjuvant therapy), radiotherapy destroys minute, microscopic cancer cells that may have remained during surgical excision [Figure 2].[20]

Radiotherapy is effective in removal of cancer cells and functions in various ways. The three distinct pathways it works are:

1. “Rapid interphase” death: Post irradiation, death occurs almost immediately
2. “Delayed interphase” death: After subjecting to radiation, death occurs only after the G2 phase
3. “Mitotic/delayed mitotic” death: Delayed death of cells after irradiation, where cells undergo one or more mitoses prior to death.

Understanding these pathways are essential, as cell death time and pattern varied in different cell lines upon irradiation. These differences are to be taken in consideration as they serve as useful indicators when treating each of these cell lines.[22]

Following radiotherapy, radioresponsive tumors show an acute apoptotic response, as apoptosis can be induced following irradiation of tumors. Apoptotic count may be valuable as a high apoptotic count was always associated with worse prognosis following radiotherapy. Numerous apoptotic cells were demonstrated in areas of high mitosis which indicated that high apoptotic count correlated with a highly proliferative tumor.[23] Factors that can predict radiosensitivity suggest a panel consisting of both pro-apoptotic as well as anti-apoptotic markers to know the balance between them, as it is very essential for a good treatment response.[23]

p53

The main target of radiation damage is the DNA of the cell. Post radiation, p53 protein dramatically increases as it senses the DNA damage. Bystander effect is a phenomenon where alteration in expression of p53 gene is elicited in those cells that do not receive direct radiation exposure. Cell-cell communication between cells via gap junctions can lead to this. Thus, p53-mediated DNA damage can be induced in irradiated tumor cells as well as bystander cells.[24]

p53 protein either arrests the cell cycle or induces apoptosis in response to radiotherapy in a dose-dependent manner. The function of p53 in cancers can be altered by numerous mechanisms: Tumors that prevent p53 activation, mutations either within the p53 gene itself or in the mediators of p53 protein. It is seen that substantial improvement of symptoms was seen when exogenous p53 was used in chemo- or radiation-resistant advanced cancers.[24]

Caspases

To enhance the efficacy of radiotherapy, caspases play a role in chemical and physical agent-induced apoptosis. They have an intense impact on macrophage activation and cytokine production. This occurs as caspases shift the balance between three components namely, apoptosis, necrosis, and autophagy. Apoptosis via the extracellular pathway requires externalization of phosphatidylserine, bleb formation, and internucleosomal DNA fragmentation. These crucial steps depend on caspase 3 activity. They produce cytokines and bring about apoptotic cell death.[25]

Inhibitor of apoptosis (IAPs)

Survivin is an anti-apoptotic marker (IAPs). Hence, increased expression of survivin protein indicates increased proliferation of tumor, with increased angiogenesis and poor treatment outcome.[26] Survivin enhances cancer cell survival by suppressing apoptosis-induced cell death, by blocking the function of caspases gene directly. This causes changes in the cell cycle and impairment in repair of DNA double-strand break. This decreases the therapeutic efficiency of radiotherapy.[26]

TNF

TNF is a multipotent cytokine that has the ability to mediate cytotoxicity without inhibiting normal cell growth. Activated macrophages produce this cytokine and it’s anti-tumor activity depends on tumor microenvironment. TNF causes selective destruction of tumor-associate angiogenesis, activation of inflammatory as well as immune mechanisms, tumor cell death, and necrosis. TNFα is highly potent and can induce apoptosis in highly resistant target cells.[20]

Endothelial growth factor receptor (EGFR)

EGFR used in combination with radiotherapy can control tumor growth compared to irradiation alone. Through modified signal transduction, it causes cellular radiosensitization and directly
destroys oncogenic cells. It repairs the DNA damage and improves reoxygenation during fractionated radiotherapy.

Inhibition of EGFR expression causes increase in the apoptotic cell death of tumor cells, overexpression lower tumor control rates after radiotherapy. Hence, EGFR expression can be used as possible prognostic marker to measure radiosensitivity.\[27\]

**Angiogenesis**

Tumor angiogenesis is important for tumor growth, invasion, and metastasis. The tumor cells trigger several growth factors and create a hypoxic environment within the tumor, which mediates angiogenesis. If apoptotic cells are present near the vasculature, it is suggestive of good prognosis, while its absence suggests that it is a highly proliferative tumor with bad prognosis.\[30\]

**Prognosis**

For an effective individualized treatment, identification of prognostic markers to assess the treatment is essential. Chemoradiotherapy delivered in combination with molecular targeted therapy will improve the prognosis of cancer patients definitely at a future date.\[32\]

**Conclusion**

New age targeted cancer therapy requires a thorough understanding of the molecular links between tumor origin, apoptotic mechanisms, and drug resistance. This will pave a new path in cancer research, diagnostics, prognosis, and therapy. There will be new therapies that target mutated cells and are nontoxic, that are better than current treatment regimens.

**References**
