INTRODUCTION

Cell proliferation and cell death maintain equilibrium between various cellular reactions such as regeneration, hyperplasia, dysplasia, hypertrophy, atrophy or metaplasia in multicellular organisms. Cell death appears to be a basic biological phenomenon which is maintained by the human body. It physiologically removes these cells, which is required for the regulation of tissue homeostasis. Alteration in molecular mechanisms involved in apoptotic signaling contributes to a vast range of oral diseases. An understanding of the regulation of apoptosis has led to the development of many therapeutic approaches and better management of oral diseases. The review updates us the correlation between apoptosis in normal oral tissues and oral diseases.

This review emphasizes on the current literature on the role of apoptosis in diseased oral tissues. An understanding of their role in the pathophysiology of oral diseases would help in the development of novel therapeutic approaches in future.

MECHANISM OF APOPTOSIS

Apoptosis is the end-point of an energy-dependent cascade of molecular events, initiated by certain stimuli and consists of four separable but overlapping components.

- Signaling pathways that initiate apoptosis: Apoptotic stimuli generate signals that are either transmitted across the plasma membrane to intercellular regulatory molecules or addressed more directly at targets present within the cell. Transmembrane signals may be positive or negative determinants of apoptosis. Transmembrane positive regulators of apoptosis are receptor–ligand interactions at the plasma membrane that generates signals to activate death program.

Key Words: Bax/B-cell lymphoma 2, caspase, death domain, precancer, programmed cell death, tumorigenesis.
Control and integration stage: This is performed by specific proteins that connect death signals to the execution program. There are two broad categories for this stage:

A. Transmission of signals by specific adapter proteins to the execution mechanism

B. Members of the B-cell lymphoma 2 (Bel-2) family of proteins which play a major and ubiquitous role in apoptotic regulation largely by regulating mitochondrial function.

Permeability transitions occur by the formation of pores and also there is a release of cytochrome C which is an apoptotic trigger. This can be inhibited by Bel-2. The Bel-2 family proteins, by selective binding, either act as antiapoptotic factors (Bel-XL) or can also promote apoptosis (Bax, Bad).

Another notable protein, the pro-apoptotic protease activating factor (Apaf-1), triggers initiator caspase (caspase-9) after it binds to the cytochrome released from the mitochondria, triggering the process of apoptosis. Bel-2 plays an important role here too, by sequestrating it and preventing cell death under normal conditions.[3,4]

The execution phase: The cysteine proteases acting here belong to the caspase family. Caspase 9 gets triggered by binding to Apaf-1 and caspase 8 by Fas–Fas ligand interaction [Figure 1]. All these exist as zymogens and undergo cleavage for triggering apoptotic cascade. Rapid and sequential autocatalytic cascade is activated following the initial event.

Removal of dead cells: Marker molecules on the surface of the apoptotic cells help in their recognition, phagocytic uptake and disposal by adjacent cells or phagocytes.[4]

An integrated schematic diagram to show the extrinsic and intrinsic factors associated with the apoptotic event is depicted in Figure 2.

Apoptosis in oral cavity in healthy individuals

PCD is described as the death of specific cells at a specific time which are destined to die during embryogenesis. It occurs in the oral epithelium as terminal differentiation where basal keratinocytes continuously proliferate, mature and differentiate to flattened squames, which are then shed off from the epithelial surfaces as keratin squames.[5]

Apoptosis-associated diseases

1. Disorders associated with prolonged cell survival
   A. Cancers including breast, central nervous system, head and neck, follicular lymphoma, involving gastrointestinal tract, genitourinary tract, lung, oral cavity and skin
   B. Autoimmune disorders such as systemic lupus erythematosus
   C. Viral infections such as herpes viruses, pox viruses and adenoviruses.

2. Diseases associated with increased apoptosis
   A. AIDS
   B. Neurodegenerative disorders such as Alzheimer’s disease and Parkinson’s disease
   C. Myelodysplastic syndromes such as aplastic anemia
   D. Ischemic injuries such as myocardial infarction and stroke
   E. Toxin (alcohol)-induced liver disease.[6]

Apoptosis in oral diseases

The mouth is the inlet to the gastrointestinal tract and is thus exposed to a wide range of ingested substances, some of which may induce physical, chemical or microbial irritation. Some individuals may react adversely to these substances by developing epithelial lesions.

Polymethyl methacrylate resins, used in removable dentures, have been shown to induce apoptosis in human monoblastoid cells and murine fibroblasts. Physical irritation from ill-fitting dentures may lead to ulceration or production of an exuberant fibrous reparative connective tissue and lesions such as epulis. Furthermore, removable dentures may contain bioactive substances that can have toxic effects on the mucosa.[6] Dentifrices commonly used in the prevention of plaque formation use triclosan as an active ingredient, which can induce cell death in human gingival epithelial cells.[7] Pyogenic granuloma is a reactive oral lesion associated with
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hormonal changes during pregnancy and is associated with complete or partial regression after childbirth. Regression of pyogenic granuloma after parturition is associated with increased apoptosis and lack of vascular endothelial growth factor.\[8\]

The other substances that may affect normal oral mucosa include tobacco products, which modulate the turnover of epithelial and mesenchymal tissues. The use of smokeless tobacco is associated with white lesions in the oral mucosa whereas smoking causes hypermelanocytosis and melanosis. Tobacco-induced changes at the cellular level may be observed in apparently normal oral mucosa of smokers after chronic exposure and include DNA aneuploidy, oxidative stress, enhanced cell proliferation and increased apoptosis.\[9\]

**Apoptosis and oral viral infections**

The ability of viruses to induce and evade apoptosis plays an important role in the pathogenesis of viral diseases. This is important as it helps in the development of vaccines which can effectively inhibit associated viral proteins.

Inflammatory response initiated by host cells protects them from these infections. Host defenses are overcome by the blockage of apoptosis by viral Bcl-2 gene or suppression of inducers of apoptosis such as p53 gene.

Herpes simplex virus induces apoptosis in activated dendritic cells which can be reduced by blocking cell death receptors but not by the antiviral drug acyclovir. Therefore, acyclovir has limited effectiveness in the treatment of herpetic infections. Infection by the varicella-zoster virus causes varicella (chickenpox) in seronegative individuals and herpes zoster (shingles) in previously infected persons. HIV infection causes apoptosis of infected lymphocytes and increased spontaneous apoptosis of uninfected lymphocytes leading to massive CD4+ T-cell depletion.\[10\]

Lymphocyte apoptosis in AIDS is induced by the viral components such as gp120 and cell surface death receptors. Oral hairy leukoplakia is characterized by massive Epstein-Barr virus replication which produces viral proteins, inducing hyperproliferation of infected cells and resists apoptosis by the production of a viral homolog of Bcl-2.\[8\] Human herpesvirus-8/Kaposi's sarcoma-associated herpesvirus associated with AIDS modulates a number of cellular events such as immune evasion, cell proliferation and inhibition of apoptosis by encoding a number of protein including viral interleukin-6 and viral Bcl-2.\[11,12\]

**Apoptosis and vesiculobullous and ulcerative diseases**

Vesiculobullous diseases are characterized by the loss of cell contact between keratinocytes or basal keratinocytes and the...
Basement membrane leading to cell death and formation of vesicles or bulla. Integrin signaling in Bcl-2-dependent pathway protects normal keratinocytes from apoptosis. Defects in this mechanism lead to few vesiculobullous diseases. Studies have shown genes such as Bcl-2, Bax and TP53 and FasL are altered in patients with pemphigus vulgaris. Mutations in keratin 5, 14 and molecular defects in hemidesmosomes involved in proliferation, apoptosis and differentiation of basal keratinocytes are associated with epidermolysis bullosa.

Ulceration of oral epithelium is common and the etiology is believed to be multifactorial including trauma, chemicals, infections, immunological disorders or neoplasms. Nonsteroidal anti-inflammatory drugs such as aspirin (acetylsalicylic acid) are widely used for the treatment of toothache that may impair mucosal defenses and tissue repair by delaying healing of oral mucosal ulcers and inhibition of cell proliferation. Topical application of aspirin induces aspirin burn.

Apoptotic degeneration of the prickle cell layer of oral mucosa was demonstrated microscopically in recurrent aphthous ulcers (RAU). More recently, lymphocytes obtained from the patients with Behcet’s syndrome associated with RAU were relatively resistant to Fas-induced apoptosis, suggesting that a defect in the Fas pathway may be involved in this condition.

Lupus erythematosus is an autoimmune disorder, showing the destruction of basal keratinocytes resulting in the formation of civatte (apoptotic) bodies. Mutations in the Fas gene have been associated with defective apoptosis in systemic lupus erythematosus.

Erythema multiforme (EM), may present as ulcers in the oral cavity, was found to be associated with altered expression of p53 and proteins of the Bcl-2 family but not the Fas/FasL system. Studies show that Fas signaling is involved in the pathogenesis of Stevens–Johnson syndrome but not in EM pointing to differences in the molecular basis of the systemic and localized forms of EM.

**Apoptosis, precancer and oral tumorigenesis**

Oral precancerous lesions are one of the most common and usually neglected lesions. However, their early detection and treatment give the best chance for its cure.

Apoptosis prevents the development of aneuploidy and other genetic abnormalities that are associated with the development and progression of precancerous lesions [Figures 3 and 4].

Soini et al. explained that tumors that exhibit less apoptosis tend to show aggressive behavior and have a greater potential for metastasis.

At certain stages during the development of a tumor, the equilibrium between the proliferation of a cell and its apoptosis is interrupted, resulting in dysregulation of cell proliferation. In benign tumors such as ameloblastoma, the role of p53, Fas, FasL and heat shock proteins in modulating apoptosis during its tumorigenesis has been found in many studies.

In odontogenic myxoma and granular cell tumor, it has been proposed that the survival of tumor cells may be attributed to suppression of apoptosis by antiapoptotic proteins of the Bcl-2 family.

Damaged normal cells due to hypoxia or anticancer therapy are eliminated by apoptosis, failure to which leads to the development of cancer. Cancer cells may evade apoptosis by inactivation of apoptosis-inducing genes or by enhancement of antiapoptosis genes. TP53 gene induces arrest of cell cycle.
and apoptosis. It exerts its effects at multiple stages of cancer progression, implying that there is a strong selection for tumor cells to inactive TP53. This is shown by the frequency with which it is mutated in human cancers.\(^{20}\)

A number of apoptosis-inducing proteins, such as Bax and BAD, may be inactivated in tumors. Bax was shown to be downregulated in oral carcinoma cells. Tumors also increase the expression of proteins that inhibit apoptosis. Bcl-XL, an antiapoptotic protein, was found to be overexpressed in oral cancer cells and could confer resistance to multiple chemotherapeutic agents in several squamous cell carcinoma cell lines.\(^{21}\)

Survivin which is not expressed in normal tissues is closely associated with expression in premalignancies and oral cancer. The selective expression of survivin in neoplastic tissues and its role in inhibition of cell death provide a novel therapeutic target for cancer treatment.\(^{5}\)

The elevated levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and lowered antioxidants have also been found to influence the rate of apoptosis in the body. The consumption of tobacco exposes the individuals to the toxic oxygen- and nitrogen-free radicals.

ROS/RNS can have following effects:
- Cause structural damage to the DNA (base-pair addition, deletion, sequence amplification, etc.)
- Cause intracellular damage (phospholipid and protein breakdown) along with cytoskeletal damage
- Affects cytoplasmic and nuclear signal transduction pathways which further dysregulates the extrinsic and intrinsic pathways of apoptosis\(^{22}\) [Figure 5].

CONCLUSION

Apoptosis has emerged over decades as an important biological process involved in normal physiology and pathogenesis of a variety of diseases.

This review is an attempt to sum up the current knowledge on the role of apoptosis in oral diseases. The understanding of the molecular basis of apoptotic cell death has led to the development of therapeutic strategies in the treatment of a number of diseases including cancer, immunological disorders and autoimmune diseases.

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