

Immunosurveillance and Oral Cancer

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INTRODUCTION

Carcinogenesis is a complex multistep process in which genetic events within signal transduction pathways that govern the normal cellular physiology are quantitatively or qualitatively altered^[1]. Oral squamous cell carcinoma (OSCC) develops as a consequence of multiple molecular events in many chromosomes and genes. The consequence of this damage is cell dysregulation with disruption in cell signaling, the cell growth cycle and/or mechanisms to repair cell damage or eliminate the dysfunctional cells^[2]. The process of carcinogenesis involves seven fundamental changes in cell physiology that determines malignant phenotype. These includes self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, defects in DNA repair, limitless replicative potential, sustained angiogenesis and ability to invade and metastasize^[3]. Another important hallmark identified for tumor development is the acquired capacity of developing tumors to escape immune control of the body. This formed the basis of immunoeediting hypothesis of tumor development.

The immune system represents a complex assortment of interacting cells and proteins which can be broadly divided into innate and adaptive types^[4]. Innate immunity refers to defense mechanisms that are present even before infection and forms the first line of defense. The major components of innate immunity are epithelial barriers, phagocytic cells, natural killer cells and several plasma proteins including proteins of complement system. Adaptive immunity consists of mechanisms that are stimulated by microbes and also recognize distinct antigens by lymphocytes carrying specific receptors. Cells of the immune system are primarily involved in adaptive immunity. Within the lymphoid system two groups of cells mainly T-lymphocytes (cell-mediated immunity) and B-lymphocytes (humoral immunity) are involved^[4,5].

Literature data accumulated over the years suggest the development of immune response to various human cancers by the host system. Humans suffering from cancers are known to spontaneously develop specific adaptive immune responses to tumor antigens through CD8 T-cells. The innate arm of the immune system

ABSTRACT

The immune system of the body plays an important role in human tumorigenesis. Literature data over the past several decades has provided evidence for the same. The ideas concerning the activities of the host immunity have evolved from the initial immunosurveillance concept which describes the anti-tumorigenic effects of the immune system to the more recently proposed immunoeediting concept. The concept of tumor immunoeediting describes the dual action of immune system in acting as tumor scavengers as well as tumor promoters. This has been described based on three stages of tumor immunoeediting namely elimination, equilibrium and escape. Oral squamous cell carcinoma (OSCC) is a multifactorial disease caused chiefly by tobacco and its products which exhibit several genetic and molecular aberrations. Among the various hallmarks of oral carcinogenesis is the role of immune system to destroy the tumor cells as well as the ability of the tumor cells to evade the host immune system. Various cells such as mast cells, tumor necrosis factor alpha (TNF- α), eosinophils, macrophages and interferon- γ have been found to be involved in OSCC. This review encompasses the basics of immunosurveillance and immunoeediting concept with a note on the different cellular mediators of immune system as pertaining to OSCC.

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may also discriminate between tumor cells and normal cells through MHC class I chain related proteins A and B which serve as ligands for NKG2D and T-cell receptors present on cell of innate immune system^[6].

The role of immune system in human carcinogenesis is often regarded as a double edged sword wherein the body's immune system is known to nullify the harmful effects of cancer cell proliferation while it may also promote tumor growth. Data available in the literature provides evidence towards the dual action of the host immune system. Recent advances in identifying various players of immune system has contributed to its current understanding that has also helped in evolution of immunotherapeutic agents as a treatment modality for cancer. This review discusses the historical perspectives, mechanism and current understanding of concepts concerning the role of immune system in carcinogenesis with additional emphasis on its role in oral squamous cell carcinoma.

HISTORY

The original description of the idea that the immune system could repress a potentially overwhelming frequency of carcinomas was envisaged by Paul Ehrlich in 1909^[7]. As the understanding of immunology expanded, Burnett and Thomas in 1957 proposed the immunosurveillance theory speculating that the immune system was capable of destroying growing malignancies^[8]. The theory speculated that the lymphocytes acted as sentinels in recognizing and eliminating continuously arising, nascent transformed cell^[9]. Subsequent experiments however disproved this concept based on the findings in athymic nude mice available for experimentation at that time^[7].

Further progression in research concerning the role of immune system in carcinogenesis led to the proposal of immunostimulation theory by Prehn (1970) who suggested that the immune system can actually promote the growth of tumors^[10].

Understanding the role of immune system in tumor rejection and tumor formation led to the modification of immunosurveillance concept into cancer immunoediting concept that describes the host protecting and tumor sculpting actions of the immune system in preventing and also shaping neoplastic disease^[7]. Cancer immunoediting comprises of three phases namely elimination, equilibrium and escape which encompasses the role of immune system in carcinogenesis^[6].

IMMUNOSURVEILLANCE IN CANCER

The primary clinical data that supported the importance of immunosurveillance as a mechanism of tumor prevention has been generated by epidemiological studies of immunodeficient patient populations^[11]. Since its conceptualization by Burnet and Thomas in 1957, several experiments have challenged its validity based on experimental findings of immunological defects in nude mice. During the recent years, significant developments have led to the renaissance of this concept based on certain key findings. An important development is the finding that endogenously produced interferon γ (IFN- γ) was shown to protect the host against the growth of transplanted tumors and the formation of primary chemically induced and spontaneous tumors^[12]. Another important event was the discovery of natural killer (NK) cells and the possibility of their functioning as effectors of immunosurveillance^[7]. The next significant finding was the role of perforin, wherein animal models lacking perforin were found to be more susceptible to tumor development^[13]. These key findings demonstrated that tumor formation is in fact controlled by host immune system. Various cellular mediators of

immunosurveillance were since being studied and the following section outlines the role of these mediators.

CELLULAR MEDIATORS OF IMMUNOSURVEILLANCE

The interferon family was originally recognized for its capacity to protect naive cells against viral infection and later was seen to have obligate roles in the elimination phase of cancer immunoediting^[14]. The action of IFN- γ in tumor rejection has been explained by different pathways. Foremost to this is the finding that the tumor cell itself is an important target in tumor rejection. Experimental findings have concluded that tumor cell is a physiologically relevant target of IFN- γ in the tumor rejection process^[15,16,17]. IFN- γ is also known to enhance tumor cell immunogenicity by upregulating components of the MHC class I antigen processing and presentation pathway which can cause tumor rejection^[18]. IFN- γ is known to have profound anti-proliferative and/or pro-apoptotic effect on certain tumor cells^[6]. Recent work suggests that $\gamma\delta$ T-cells are an important source of IFN- γ during the development of protective anti-tumor response^[19]. It was also found that host cells are important targets of IFN- γ during the development of protective anti-tumor immune response. Studies have shown that IFN- γ in combination with granulocyte monocyte-colony stimulating factor (GM-CSF) controls chronic infection thus reducing tumor development^[20]. Another possible mechanism is that IFN- γ can induce angiostatic effects in tumors by targeting non-transformed host cells that are in close proximity to the tumor^[21]. Other cells which have a role in tumor immunosurveillance include macrophages and dendritic cells, natural killer cells, B-lymphocytes and T-lymphocytes. Dendritic cells function by upregulating MHC class I and class II molecules on recognition of pro-inflammatory proteins elaborated by tumors and also upregulate CD80, CD86 and CD 137^[22]. Macrophages are efficient phagocytic cells which have immunosurveillance ability as well as promote tumor growth. Macrophages operate to both directly destroy tumors and augment the functions of natural killer cells and T-cells^[11]. Natural killer cells represent cellular populations of the innate immune compartment that protects the host from tumor formation. Robust infiltration of NK cells is nearly always associated with a favorable prognosis^[23].

CANCER IMMUNOEDITING

Experimental evidence suggests a dual role of immune system as host protecting as well as tumor sculpting effects on developing tumors. This led to the discontinuation of the term cancer immunosurveillance and replaced by the term cancer immunoediting proposed by Dunn *et al* (2004)^[6] to appropriately emphasize the dual role of host immune system in preventing and also shaping neoplastic disease. The process of cancer immunoediting has been explained based on three phases namely elimination, equilibrium and escape.

The elimination phase represents the original concept of immunosurveillance. Immunological rejection of a developing tumor requires an integrated response involving innate and adaptive immunity^[6]. Structures on the transformed cells are recognized by NK cells, $\gamma\delta$ T cells, IL-23, which are then stimulated to produce IFN- γ that is critical for progression of anti-tumor immune response^[24]. Further process involves limited tumor death produced by anti-proliferative and apoptotic mechanisms activated by IFN- γ killing of tumor cells by mechanisms involving tumor necrosis factor- related apoptosis ligand (TRAIL), perforin and reactive O₂ and N₂ intermediates and finally complete elimination of remaining

antigen bearing tumor cells by cytotoxic T-lymphocytes.

In the equilibrium phase, which is the longest, the host immune system and any tumor cell variant that has survived the elimination phase enters into a state of dynamic equilibrium. In this phase, the lymphocytes and IFN- γ exert potent and relentless selection pressure on the tumor bed that possesses many genetically unstable and mutating tumor cells.

The final escape phase is characterized by survival of tumor cell variants that have acquired insensitivity to immunological detection and /or elimination and thus begin to expand in an uncontrolled manner. Tumor cells dysregulate immune system by various local and systemic mechanisms. The local mechanism includes prevention of immune recognition by decreased tumor antigenicity, selectively attracting tumor promoting agents and rejecting anti-tumor agents, alteration in T-cell signaling, tryptophan metabolism and proteoglycans. The systemic mechanisms includes the presence of self-tolerance and regulatory T-cells, changes in dendritic cell function, myeloid derived suppressor cells, cytokines and vascular growth factors^[11].

ROLE OF IMMUNOLOGICAL CELLS IN ORAL CARCINOGENESIS

The role of immunological cells such as tumor necrosis factor related apoptosis inducing ligand (TRAIL), NK cells, dendritic cells, macrophages, eosinophils and mast cells were studied in oral cancer with an aim to understand its role in tumor progression. Oral squamous cell carcinoma (OSCC) is a multistep malignancy caused primarily by tobacco. The progression of OSCC is dependent on tumor cells themselves, the stromal microenvironment and its components. Evidence indicates that modulation of stromal cells in tumor microenvironment has a significant bearing on tumor progression. The stromal cells that were commonly studied are mast cells, macrophages, eosinophils and angiogenic factors. This section compiles the various literature data pertaining to these cells.

TRAIL is a type II transmembrane protein which plays an important role in immunosurveillance against tumor progression by selectively inducing apoptosis in tumor cells and not in normal cells^[25]. The loss of TRAIL in oral pre-malignancies and OSCC is being associated with poorer prognosis and tumor progression. Significantly high levels of TRAIL were found in normal oral mucosa than in oral pre-malignancies and OSCC^[26]. The study concluded that the loss of TRAIL expression is an early event during oral carcinogenesis and may be involved in dysregulation of apoptosis and contribute to molecular carcinogenesis of OSCC.

Mast cells display a diversity of roles in extracellular matrix degradation, angiogenesis, and innate and adaptive immune responses due to their ability to release specific products such as chymase, basic fibroblast growth factors, interleukins, TNF- α and chemokines^[27]. Local depletion of resident mast cells and a failure in mast cell migration was observed in a study by Oliveira-Neto *et al* (2007)^[27] who concluded that the decrease in mast cells might be related to the failure in migration and/or local depletion of these cells. The overall impact of decreased mast cells may reflect an important modification in the microenvironment during tumorigenesis making it more permissive to epithelial neoplastic cell proliferation. Ankle *et al* (2007)^[28] showed increased mast cell count in leukoplakia, oral submucous fibrosis, lichen planus and OSCC. Various mediators released from the mast cells might be responsible for the histological changes seen in these lesions. Gomes *et al* (2008)^[29] in their study showed significant increase in mast cells density

in lip SCC and in dysplastic actinic cheilitis than in normal oral mucosa. Increase in mast cell count may suggest an immunological cause and the density may decrease in high grade carcinoma which could be due to an unfavorable cellular environment. A transition from increase to decrease number of mast cell in oral mucosa represents a competition between the immunological system and the tumor cellular environment. Iamaroon *et al* (2003)^[30] found linear increases in mast cell count from normal oral mucosa, hyperkeratosis, and epithelial dysplasia to invasive OSCC. They also found a significant correlation between mast cells and microvascular count in OSCC. These findings suggest that mast cells may upregulate angiogenesis in OSCC via the release of mast cell tryptase. The number of mast cells and microvessels may also be used as indicators for disease progression. Rojas *et al* (2005)^[31] demonstrated that mast cells were activated and significantly increased in lip SCC than in normal lip. Significant changes in subpopulations were also found. Mast cell containing chymase and tryptase was higher at the peritumoral stroma which plays a role in extracellular matrix degradation and tumor progression at the invasive front while mast cells tryptase was increased in intratumoral stroma which may stimulate angiogenesis. Changes in proportion of mast cell tryptase to mast cell tryptase-chymase could be a useful indicator of malignancy in lip biopsies.

CD8 cytotoxic T-lymphocytes are key effectors in the antitumor immunity while CD4 T-cells play a role in antibody production and activation of CD8 T-cells and macrophages. A study by Ali *et al* (2012)^[32] did not demonstrate any significant correlation between CD4, CD8 levels and clinical parameters. Other studies found a significant correlation between CD4 and CD8 levels with tumor progression and peritumoral inflammatory infiltrate^[33,34,35].

Tumor associated macrophages are known to play a pro-tumor role in OSCC and tumor progression likely occurs through activation of the Gas 6/Axl-NF-kB signaling pathway^[13]. Li *et al* (2002)^[37] identified that tumoral accumulation of the macrophages is associated with stage of tumor invasion. Sica *et al* (2006)^[38] in their study reported predominance of macrophages in the peritumoral infiltrate in OSCC. Several studies did not find any statistical significant difference between infiltrated macrophage and the pathological stage and grade of OSCC^[32,39] (Table 1).

IMMUNOTHERAPY IN HEAD AND NECK CANCER

Development of immune based cancer therapies is difficult due to the fact that the active tumor escapes from the host immune system and/or the failure of immune surveillance to control tumor progression. Thus immune based therapies are developed to involve multiple components of the body immune system and the drug trials are based on T-cell immunotherapy, modified autologous tumor cell vaccine, dendritic cell based vaccine, DNA vaccines, development of monoclonal antibodies and cytokines^[40]. Some of the clinical trials currently in progress or completed are; multikine which is a leukocyte interleukin injection developed by CEL-SCI and currently in the stage III trial of head and neck cancer (www.cel-sci.com); phase II clinical trial of allovectin-7 which is a experimental gene based immunotherapy (NCT00050388)^[41]; effect of microsphere delivered IL-2, IL-12, GM-CSF in HNSCC conducted by New York University school of medicine (NCT00899821)^[42]; phase I trial of TLR8 agonist VTX-2337 and cetuximab in treating patients with HNSCC conducted by University of Washington (NCT01334177)^[43]; phase I trial of vaccine therapy in stage IV HNSCC using recombinant

Table 1 Literature data on study of immune cells in oral squamous cell carcinoma.

Sl. No	Author	Study design	Results	Inference
1	Vigneswaran <i>et al</i> ^[26] (2007)	Expression of TRAIL, a type II transmembrane protein in oral epithelial cell lines and archival tissues of pre-malignancies and OSCC	Significantly low levels of TRAIL in oral pre-malignancies and OSCC than normal mucosa	Loss of TRAIL expression may be involved in dysregulation of apoptosis and is associated with poor prognosis.
2	Oliveira-Neto <i>et al</i> ^[27] (2007)	Evaluation of mast cell density and migration in OSCC and leukoplakia and their correlation with clinical and microscopic parameters	Decrease in mast cell count in pre-malignant and malignant tissues than in normal controls.	Decrease in mast cells count may be related to migration failure which could be an important modification in tumor microenvironment leading to epithelial neoplastic proliferation
3	Ankle <i>et al</i> ^[38] (2007)	Mast cell count in tissue samples of leukoplakia, oral submucous fibrosis, lichen planus and OSCC	Increase in mast cell count in diseased individuals than normal	Mediators released from mast cells may be responsible for histological changes in these lesions
4	Gomes <i>et al</i> ^[29] (2008)	Mast cell density in lip SCC and dysplastic actinic cheilitis	Increase mast cell count in disease than normal controls	Suggests an immunological cause and the decreased count in high grade carcinoma could be due to unfavourable cellular environment.
5	Iamaroon <i>et al</i> ^[30] (2005)	Mast cell count in normal mucosa, hyperkeratosis, epithelial dysplasia and OSCC	Linear increase in mast cell count from normal oral mucosa to invasive carcinoma	Mast cell may upregulate angiogenesis via release of mast cell tryptase.
6	Rojas <i>et al</i> ^[31] (2005)	Mast cell count in lip SCC and normal mucosa	Significantly increased levels in lip SCC than in normal lip	Different subpopulations of mast cells such as chymase and tryptase play specific role in oral carcinogenesis
7	Ali <i>et al</i> ^[32] (2012)	Correlation between CD4, CD8, and clinical parameters in archival tissues of OSCC	No significant correlation amongst the three parameters and with clinical features	Suggest a role for CD4, CD8 in OSCC
8	Li <i>et al</i> ^[37] (2002)	Immunohistochemical expression of CD68 labeled macrophages in OSCC	Significantly high number of CD68 positive cells noted in oral cancer	Tumoral accumulation of macrophages is associated with stages of tumor invasion. Tissue associated macrophages may play a role in angiogenesis.
9	Debta P <i>et al</i> ^[50] (2011)	Mast cells and tissue eosinophils in OSCC	Increased infiltration of tissue eosinophils and mast cells in OSCC	Favorable prognosis in patients showing increased mast cells and tissue

fowlpox-TRICOM vaccine (NCT00021424)^[44]; phase II clinical trial of safety of IRX-2 drug in head and neck cancer (NCT00210470)^[45]; phase I clinical trial of recombinant interleukin-15 in HNSCC (NCT01727076)^[46]; an interventional study of ADXS 11-001 in HPV induced oro-pharyngeal SCC (NCT02002182)^[47]; phase II clinical trial of neoadjuvant immunotherapy in OSCC^[48] and phase II clinical trial of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor in oral squamous cell carcinoma^[49].

CONCLUSIONS

Human immune system contributes significantly to head and neck carcinogenesis. The concept of immune system influencing various aspects of human cancer has been studied a great deal in the past. However its actual role is still debatable as to whether they promote tumor growth or have tumor inhibiting actions. The immunosurveillance concept was proposed based on the fact that the body immunity destroyed tumor cells and prevented the tumor from occurring. Over time, the concept of immunoediting was given importance which accounted for the dual role of host immunity in carcinogenesis. Various cellular mediators of immunity were studied with an attempt to better understand its role in carcinogenesis. Interferon- γ , mast cells, TNF- α , tumor associated macrophages and eosinophils were evaluated in OSCC but not much has been proved. A more comprehensive assessment is required to prove the role of these immune cells in OSCC. This is necessary in view of implementing immunotherapy as a therapeutic measure in the treatment of OSCC.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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