Review Article

Molecular concept in human oral cancer

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ABSTRACT

The incidence of oral cancer remains high in both Asian and Western countries. Several risk factors associated with development of oral cancer are now well-known, including tobacco chewing, smoking, and alcohol consumption. Cancerous risk factors may cause many genetic events through chromosomal alteration or mutations in genetic material and lead to progression and development of oral cancer through histological progress, carcinogenesis. Oral squamous carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal functions of proto-oncogenes/oncogenes and tumor suppressor genes. Furthermore, these gene alterations can deregulate the normal activity such as increase in the production of growth factors (transforming growth factor-α [TGF-α], TGF-β, platelet-derived growth factor, etc.) or numbers of cell surface receptors (epidermal growth factor receptor, G-protein-coupled receptor, etc.), enhanced intracellular messenger signaling and mutated production of transcription factors (*ras* gene family, *c-myc* gene) which results disturb to tightly regulated signaling pathways of normal cell. Several oncogenes and tumor suppressor genes have been implicated in oral cancer especially cyclin family, *ras*, PRAD-1, cyclin-dependent kinase inhibitors, p53 and RB1. Viral infections, particularly with oncogenic human papilloma virus subtype (16 and 18) and Epstein-Barr virus have tumorigenic effect on oral epithelia. Worldwide, this is an urgent need to initiate oral cancer research programs at molecular and genetic level which investigates the causes of genetic and molecular defect, responsible for malignancy. This approach may lead to development of target dependent tumor-specific drugs and appropriate gene therapy.

Key words: Growth factor, oncogenes, oral cancer, transcription factor

Introduction

Cancer is one of the most common cause of morbidity and mortality, however, approximately >10 million newly diagnosed and >6 million deaths occur each year due to cancer. Oral cancer is the sixth most common cancer in the world.[1,2] According to a report of Government of India, approximately 2–2.5 million cases of cancer with around 7–9 lakhs new cases being diagnosed every year.[3] Worldwide, the highest number of oral cancers with up to 80,000 new cases annually diagnosed in India.[4]

The International Classification of Diseases defines that oral cancer as cancer of the oral cavity and pharynx, including cancer of the lip, tongue, salivary glands, gum, floor and other areas of the mouth, oropharynx, nasopharynx, hypopharynx, pharynx and other buccal areas. The oral squamous cell carcinoma (OSCC) represents >90% of malignant neoplasms of the mouth.[5,6]

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Cancer of tongue is the leading site not only among oral cancers but also among head and neck cancers in India and across, however prevalence of buccal mucosa cancer is also high in North Indian population.[7,8] Lifestyle and environmental factors have been identified as the risk factor for oral cancers, tobacco chewing along with smoking and alcohol consumption are widely considered to be major risk factors for oral cancer.[9] Viruses mostly hepatitis viruses, human papilloma virus (HPV), and Epstein-Barr virus (EBV) are also linked to cancer, strongest associations have been reported between HPV and squamous cell carcinoma of the oral cavity and oropharynx. There is sufficient evidence for a causal role of HPV-16 in OSCC, but it is limited for HPV-18 type.[10-14] Some bacteria such as Streptococci species, *Staphylococcus aureus*, *Veillonella parvula*, *Micrococcus* species along with species of *Candida* are significantly related to human mucosal carcinoma.[15,16]

Worldwide, the major problem is to identify the cause of oral carcinoma in individuals without any bad habits like tobacco chewing/smoking and alcohol consumption. Hence, it is urgent need to identify other features like poor oral hygiene or dental status, oncogenic viruses and genetic predisposition which may associate with the occurrence of oral cancer.[8]

Cancerous risk factors may cause many genetic events through chromosomal alteration or mutations in genetic material and lead to progression and development of OSCC. This has previously been reviewed on the molecular biology of human oral cancer.[10,17-20] This review is to give a basic and current knowledge about genetics and molecular biology of oral cancer in human. This molecular level approach for understanding oral cancer may expose new targets to identify events in the carcinogenic process and propose to opportunities for treatment/prevention of oral cancer.

Oral Carcinogenesis

In general, every eukaryotic cell of human contains genes which are composed of a double-stranded coiled molecule known as deoxyribonucleic acid (DNA), located within the nucleus as 23 pairs of chromosome. The nucleotides sequences of DNA produce different types of protein which act as effectors molecule as well as participated in many different types cellular functions. In normal conditions, various tightly controlled excitatory and inhibitory pathways regulate to oral epithelial cell biology such as cell division, differentiation, and cell death (apoptosis). An extracellular ligand like growth factor (a protein) binds with a specific cell surface receptor. The receptor-ligand complex generates excitatory or inhibitory signals sent through intracellular and nuclear messengers that can either alter cell function by changing the effect of proteins.[21] Carcinogenesis is a complex, multi-step process in which genetic events within signal transduction pathways are subverted/altered resulting enhance to cell's ability for proliferation, uncontrolled apoptosis or grow by invading locally or metastasizing to distant sites. The histological progression of oral carcinogenesis is believed to reflect the accumulation of these changes [Figure 1].[19,22-24]

Cytogenetics in Human Oral Cancer

Boveri stated about tumor cell chromosomes and alterations in these chromosomes resulted in conversion of normal to malignant proliferation.[25] Chromosomal instability is a common feature of human tumors including oral cancer. There are many causes of chromosomal instability, although the primary causes appear to be defects in chromosomal segregation, telomere stability, cell cycle regulation and the repair of DNA damage.[26] More than 63 karyotypes have been described in human oral cancer in which recurrent loss of chromosome 9, 13, 18 and Y deletions are more common. Frequent deletion of the chromosomal region involved in arms of 3p, 7q, 8p, 1lq, 13q, and 17p and in the short arm of all acrocentric chromosomes may yield oral carcinogenesis.[20,27] Approx. two-third of all head and neck cancer cells contain a deleted region located in chromosome 9p21-22.[28] Cells of oral cancer can be divided into two types in reference to genetic damage; Dominant changes mostly occurs in proto-oncogenes but also in certain tumor suppressor genes (TSGs) resulting in a gain of function. Recessive

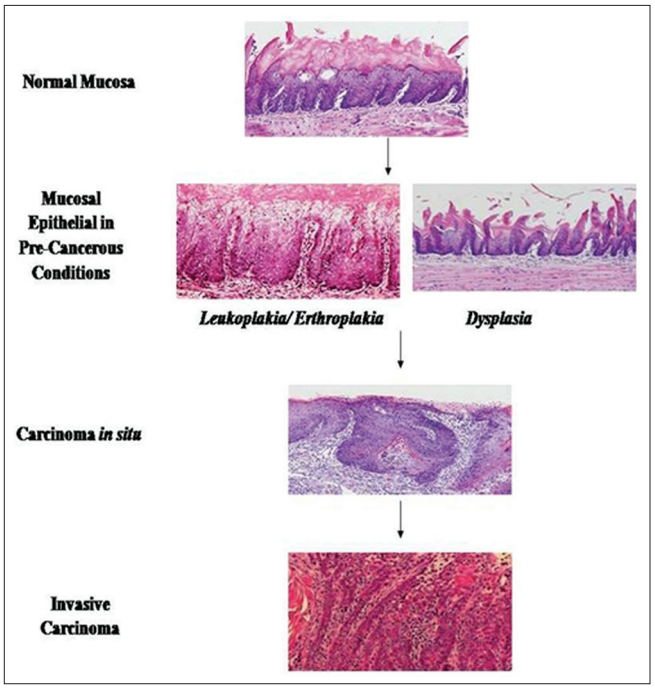

Figure 1: Histological progression of oral carcinogenesis

changes can be occurred by mutations most frequently in growth-inhibitory pathway genes or commonly in TSGs and causes loss of function.[21,29]

Oncogenes

Initially, oncogenes were identified in retroviruses causing cancer. A series of studies by Peyton Rous on fibrosarcomas (connective tissue tumor) of chickens reported that *Sarcoma virus* (a retrovirus) developed to the tumor. Ultimately, Rous was awarded the Nobel Prize in 1966 for his pioneering work.[30] An oncogene is any gene that encodes a protein able to transform cells and induce cancer in human or animal. There are two broad classes of genes; proto-oncogenes and tumor-suppressor genes which play a key role in cancer progression. These genes encode many kinds of proteins that help control cell growth and proliferation but mutations in these genes can contribute to the development of cancer.[31]

Of the many known oncogenes, few are derived from normal cellular genes (i.e., proto-oncogenes) whose products participate in cellular growth-controlling pathways. Conversion or activation of proto-oncogenes into an oncogene generally involves a gain-of-function mutation. At least three mechanisms can produce oncogenes from the corresponding proto-oncogenes: (i) Point mutations in a proto-oncogenes that result in a constitutively acting protein product, (ii) localized reduplication (gene amplification) of a DNA segment that includes a proto-oncogenes leading to overexpression of the encoded protein, (iii) chromosomal translocation that brings a growth-regulatory gene under the control of a different promoter and that causes inappropriate expression of the gene. Several cellular oncogenes are homologs of retroviral oncogenes (e.g., the *ras* genes) and have been implicated in oral carcinogenesis.[22,32] Unusual expression of the proto-oncogene like epidermal growth factor receptor (EGFR), members of the *ras* family, c-myc, *int-2*, *hst*, PRAD-1, and *bcl* is believed to participated to oral cancer development [Table 1].[33-35]

Table 1: Some well-known oncogenes and their encoded proteins

Oncogene	Properties of protein	
	Location	Function
Nuclear transcription regulators		
jun	Nucleus	Transcription factor
fos	Nucleus	Transcription factor
erbA	Nucleus	Member of steroid receptor family
Intracellular signal transducers		
abl	Cytoplasm	Protein tyrosine kinase
raf	Cytoplasm	Protein serine kinase
gsp	Cytoplasm	G-protein α subunit
ras	Cytoplasm	GTP/GDP-binding protein
Mitogen		
sis	Extracellular	Secreted growth factor
Mitogen receptors		
erbB	Transmembrane	Receptor tyrosine kinase
fms	Transmembrane	Receptor tyrosine kinase
Apoptosis inhibitor		
Bcl 2	Cytoplasm	Upstream inhibitor of caspase cascade

GTP: Guanosine triphosphate, GDP: Guanosine diphosphate

Growth Factors and Cell Surface Receptors

Mostly, the seven types of proteins are participates in controlling of cell growth: (i) Growth factors, (ii) growth factor receptors, (iii) signal-transduction, (iv) transcription factor, (v) pro-or anti-apoptotic proteins, (vi) cell cycle control proteins, and (vii) DNA repair proteins.[36] Cancer can result from the expression of mutant forms of these proteins. For example, growth factor (a type of extracellular ligand) binds to a cell surface receptor. The receptor-ligand complex generates excitatory or inhibitory signals sent through intracellular and nuclear messengers that can alter cell function by changing the effect of proteins. During oral carcinogenesis, growth factors are de-regulated through increased production and autocrine stimulation [Figure 2].[32,37]

Transforming growth factor- α (TGF-α) is overexpressed early in oral carcinogenesis by hyperplastic epithelium and later by the inflammatory infiltrate particularly the eosinophils, surrounding the invading oral epithelium. However, other protein of this gene family TGF-β is recognized for its tumor suppressor as well as tumor promoter activity.[38-40] In carcinoma, tumor cell expressed to platelet-derived growth factors and led to an autocrine mechanism that drives carcinogenesis and tumor progression.[41]

Cell surface receptor binds to its specific ligands and activates a cascade of intracellular biochemical steps, and regulation of protein phosphorylation is an important event in cellular function and gene expression. Alteration/mutation in genes encoding to cell-surface receptors can result in an increased number of receptors or production of a constituent ligand-independent mitogenic signal.[42-44] EGFR is a cell surface (170,000 dalton) tyrosine kinase transmembrane receptor along with a member of the human EGFR (HER)-ErbB family. The ErbB family is composed of four transmembrane receptors that interact with each other: EGFR/ErbB1/HER1, ErbB2/HER2/neu, ErbB3/HER3, and ErbB4/HER4.[45]

The interaction between ligand and its specific receptor can result in either homodimerization or

heterodimerization of receptor and ligand complex, then intracellular tyrosine kinase portion is phosphorylated and leading to downstream activation of complex interacting signaling pathways which include the Ras/Raf/MEK/ERK and the Ras/PI13K/PTEN/AKT/mTOR pathways. EGFR and its biological ligands (EGF, TGF-α) are frequently found to be overexpressed in human oral cancers.[46-49]

Recent studies reported, G-protein-coupled receptors (GPCRs), the largest family of cell-surface molecules involved in signal transmission and plays a crucial role in tumour growth and metastasis.[50] The specific members of GPCRs family involved in the transmission of signals from the extracellular environment to the cytoplasm. A wide variety of chemical or physical stimuli can activate and promote interaction between the GPCRs and the G-protein on the intracellular side of the membrane. GPCRs comprise the largest integral membrane protein family in the human genome with over 1000 members and grouped into 5 classes.[51,52] In all these classes, adrenergic receptors (ARs) are kept in amine group of class A receptors and are composed of two main subfamilies, α and β, which differs in tissue localization and ligand specificity, as well as in G-protein coupling and downstream effectors mechanisms. Each subfamily further sub grouped in - α1, α2 and β1, β2-adrenergic receptor.[53,54] In some recent studies, the expression of β2-AR in breast cancer, colon cancer, and prostatic cancer. An *in-vitro* experiment (by Wan *et al*.) on mutant S49 mouse lymphoma cells proved to activation sequence of a GPCR by its specific ligands and downstream effectors molecules, as β-adrenergic receptor/Gsα/adenylyl cyclase/cAMP/PKA/Rap1/B-Raf/MEK/MAPK [Figure 3].[55-59]

Second Messengers and Transcription Factors

Commonly intracellular signaling molecules called as second messenger, released by the cell. Second messengers are relay to signals received at receptors on the cell surface such as the arrival of protein hormones, growth factors, etc. to target molecules in the cytosol and/or nucleus and trigger to physiological changes like proliferation, differentiation, migration, survival, and apoptosis. The cell releases second messenger molecules in response to exposure to extracellular signals first messengers (extracellular substance).[60,61] Second messengers can also be intrinsically activated, thereby delivering a continuous rather than a ligand regulated signal.[43] Of all the members of the intracellular signaling pathway, only members of the *ras* gene family (H-ras, K-ras, N-ras) have been examined in human oral cancer. They all encode for the related protein p21 that has been localized to the cytoplasmic side of the cellular membrane and which transmits mitogenic signals by binding guanosine triphosphate (GTP). The mitogenic signal is terminated by the conversion of GTP to guanosine diphosphate by hydrolysis but when the *ras* oncogene is mutated, this conversion can be prevented thus leading to continuous stimulation.[36]

Some proteins are participated a crucial role in activation of other gene, known as transcription factors. These transcription factors activity and regulation mechanisms may alter in oral cancer. The functional activity of many of these proteins is regulated by receptor activated second

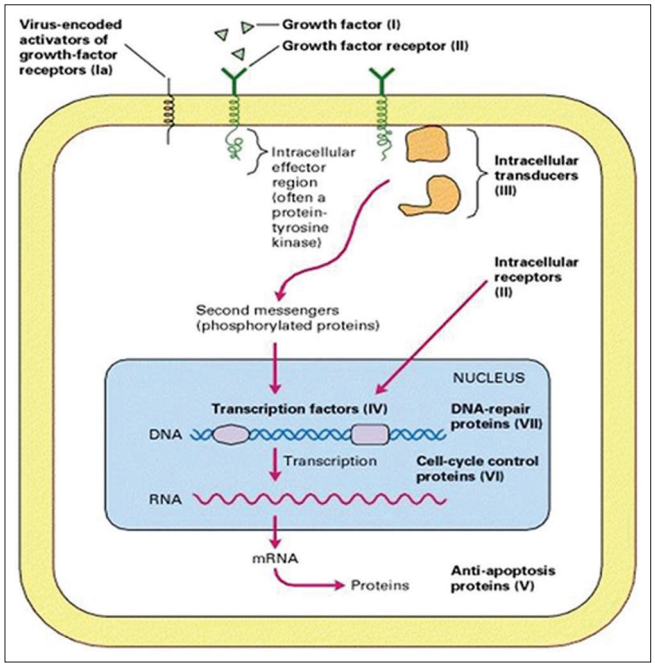

Figure 2: Signal transduction pathway of growth factor (a type of extracellular ligand)

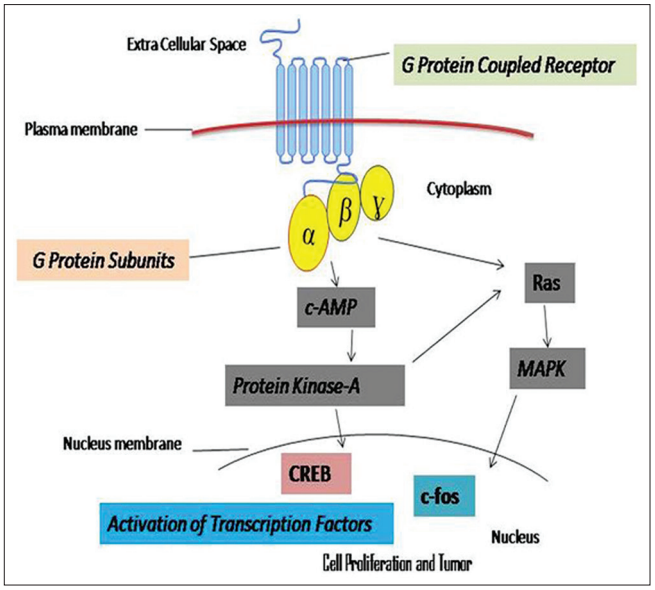

Figure 3: A diagrammatic representation of signal transduction mechanism of G-protein-coupled receptor

messenger pathways, and neutralization of these genes could result in a cell cycle block, preventing mitogenic and differentiation responses to growth factors. Among these genes, *c-myc* which helps regulate cell proliferation and frequently overexpressed in oral cancers as a result of gene amplification.[34,62] The transcriptional factor c-myc can induces both cell proliferation and apoptosis. TSG p53 induces apoptosis by activation of c-myc and another gene Rb-1 (a TSG). Nuclear protein pR6 interacts with the *c-myc* gene and preventing the transcription, inhibits cell proliferation. c-myc, p53, and pR6 are expressed in all oral carcinomas, irrespective of differentiation.[63,64] Transcriptional factor PRAD-1 (also known as cyclin D1 or CCND1) encoded by PRAD-1 gene, which together with the Rb gene product controls the G1 to S transition of the cell cycle and act as cell cycle promoter. The PRAD-1 gene is amplified in 30–50% of head and neck cancers.[65]

Tumor Suppressor Genes

Only oncogenes alone are not responsible to cause oral cancer. The transformation of a premalignant cell to a malignant cell is inactivation of cellular negative regulators which called as TSGs. TSGs is regarded to be the development of malignancy and most often inactivated by point mutations, deletions, and rearrangements in both gene copies.[66] The TSG p53 is known to be mutated in approximately 70% of adult solid tumors. p53 protein blocks cell division at the G1 to S boundary, stimulates DNA repair after DNA damage and also induces apoptosis. These functions are achieved by the ability of p53 protein to modulate the expression of several genes like WAF1/CIP gene which encode p21 protein. p21 acts as an inhibitor of cyclin and cyclin-dependent kinase complexes.[23,67-69] Smoking and tobacco use have been associated with the mutation of p53 gene in head and neck cancer.[70] Another TSGs, doc-1 gene is mutated in malignant oral keratinocytes and lead to a reduction of gene expression and protein function.[71]

Implication of Viruses in Oral Cancer

A number of viral agents have been linked to human tumors along with oral epithelial dysplasia and squamous carcinoma. Among these, HPV holds a prominent position, there are more than 77 types have been identified and categorized on the basis of differences in DNA sequences. HPV types 2, 6, 11, 16, 18, 31, 33, 35 have been detected in oral epithelial dysplasia. Approximately 33–50% of OSCCs are infected with HPV-16 and 18.[10] HPV virus contains two gene products (E6 and E7). E6 or high-risk HPVs are capable of forming specific complexes with vital cell-cycle regulators which binds to p53 and induces its degradation. E7 or low-risk HPVs interacts with pRb and blocks its downstream activity. Functional de-regulation of these key of oncosuppressors results in uncontrolled DNA replication and apoptotic impairment and explains the increased tumorigenic ability of high-risk types.[72,73] Some other viruses, a member of the herpes virus family known as EBV has been more frequently detected in oral lesions such as oral lichen planus and OSCC in comparison with healthy oral epithelium. Hepatitis C virus infected has been identified in oral lichen planus patients. 1–2% of the patients with oral lichen planus develop squamous cell carcinoma of the oral cavity, which implies the existence of common pathogenic mechanisms among them.[74-76]

Conclusions

In past few years, the development of molecular biology techniques and genetic level approaches has opened up a new dimension in our understanding to oral cancer. This understanding has disclosed new vista for the development of effective therapies as well as control and management of oral cancer. This review has been highlighted about carcinogenesis, proto-oncogenes, oncogenes, TSGs, viruses, transcription factors, and some signaling pathways which play a crucial role in progression and development of oral cancer. Multiple genetic events are responsible for carcinogenesis process such as the activation of oncogenes and inactivation of TSGs. Hence worldwide, this is an urgent need to initiate oral cancer research programs at molecular and genetic level which investigate to alterations responsible for malignancy. It may be a realistic expectation that early prevention of oral cancer will be possible and this approach may lead to development of target dependent tumor-specific drugs and appropriate gene therapy.

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Conflicts of interest

There are no conflicts of interest.

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