Review Article

University J Dent Scie 2023; Vol. 9, Issue 1

Understanding the role of Molecular and Genetic Alterations in Proliferation and Progression of Oral Squamous Cell Carcinoma : A review article.

Abstract:

Oral carcinogenesis is known as multifactorial process which engaged plentiful genetic events that transform normal activity of tumor suppressor genes and oncogenes. It is observed that aggregation of genetic alterations is the ground for advancement of a normal cell to cancer cells, which is known as a multi-step carcinogenesis. Because of this event, growth factor production increases as well as increase in total of receptors on cell surface and increased intracellular signal messengers. The present review scrutinize the existing documentation in the literature related to the oral squamous cell carcinoma. English language articles were searched in various databases such as Pubmed, Scopus, Science direct and Google scholar. The keyword used for searching are “oral squamous cell carcinoma”, “Genetics and Oral squamous cell carcinoma”,“Molecular mechanism in oral squamous cell carcinoma”. The present review spotlights on understanding the molecular mechanism and the genetic factors which is responsible for alteration in the cell which leads to oral squamous cell carcinoma.

Key-words: Oral squamous cell carcinoma, Genetic alterations, Molecular mechanism, Proliferation, Signaling

Introduction:

The squamous cell carcinoma is the most ubiquitous malignancy seen in the head and neck, pharynx, and oral cavity. Generally,3 lakh new oral cancer cases, as well as roughly 68 thousand loss of life worldwide are predicted annually[1]. Squamous cell carcinoma is defined as "a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges"[2].

In modern years, a considerable breakthrough has been made in recognition of the genetic and molecular basis of the advancement of oral squamous cell carcinoma. According to multi-step carcinogenesis, aggregation of genetic alterations in the ground for growth from a normal cell to a cancer cell[3]. Progression or advancement is facilitated by more and more abnormal functions of genes that positively as well as negatively balanced the condition of proliferation, apoptosis, angiogenesis, genome stability, invasion, and metastasis. There are various ways by which gene function can be transformed like oncogenes can be activated by amplification or mutation and tumor suppressor genes may be inactivated by deletion, mutation, or methylation[4,5,6].

Access this article online

Website: www.ujds.in

Quick Response Code

DOI: https://doi.org/10.21276//ujds.2023.9.1.17

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Received : 27 July., 2022, **Published :** 31 March, 2023

How to cite this article: Singh, H., Patel, V., Sharma, D., Sharma, N., Razdan, R. A., & Parihar, N. (2022). Understanding the role of molecular and genetic alterations in proliferation and progression of oral squamous cell carcinoma : A review article. UNIVERSITY JOURNAL OF DENTAL SCIENCES, 9(1). 83-91

Universal alterations for oral cancer include TP53 inactivation, the progress of chromosomal material at 3q26 as well as 11q13, and losses at 13q21,3p21, and 14q32[7]. The tumor microenvironment is considered an essential contributor to fostering proliferation, survival, neoplastic process, and migration[8].

Recent experiments have developed the perception that tumor microenvironments are the important components of the progression of tumors along with inflammation and hypoxia. Plentiful cancers arise from the location of inflammation, chronic irritation, and infection. Some tumors also consist of hypoxic microenvironments which are related to poor prognosis as well as resistance to treatment[9,10]. Hence, oral carcinogenesis is an eminently complex multifactorial process that occurs when several genetic alterations affect the epithelial cells.

Molecular Genetics/genetic Alterations

P16/cyclin D1/prb/p53

p16 and p53 are two crucial tumor suppressor proteins that are intermittently inactivated in head and neck cancer.p16 is encoded by the CDKN2A gene which is positioned on chromosome 9p21.p16 is implicated in cell growth as well as cell cycle control. Individually p16 blocked the growth from G1 to S phase of the cell cycle by inhibiting a separate protein known as Cyclin D1. Hence, interruption of activity of p16 outcomes in loss of cell senescence afterward leads to dysplasia[11,12].

The p53 is a tumor suppressor gene having a molecular weight of 53kDa.The p53 gene encodes a protein that seized the cell cycle at the late G1 phase in those cells which have sub-lethal impairment in their genome as far as their entire repair or it induces apoptosis in unrecoverable injury cases[13,14]. Mutations of the p53 gene are scattered over a considerable hundred base pairs in the mid-region of the gene. They occur especially in exons 5-8, which are recognized as hot regions. During metastasis, these mutations remain steady. Mutation of the p53 gene appears during the early phase or stages in the progression of head and neck squamous cell carcinoma for the reason that they are earlier present in premalignant lesions[15,16].

Cyclin D1 protein is encoded by gene CCND1, whose activity is constrained by p16. It is observed that the CCND1 gene is amplified in approximately 25-43% of oral squamous cell carcinoma[17,18]. Cyclin D1, which is the product of the CCND1 gene, was seen to be overexpressed during the early phase of oral carcinogenesis. Also, Cyclin D1 is found to be associated with tumorigenic proliferation[19,20]. It was observed that expression of cytoplasmic Cyclin D1 was increased in oral squamous cell carcinoma with advanced stages, increased mitosis, poor differentiation, and invasive cell morphology, implying that increased level of cytoplasmic Cyclin D1 encouraged cell migration and invasion. Furthermore, in some studies, Cyclin D1 expression and CCND1 amplification were associated with poor prognosis and decreased rate of survival[21,22].

Notch:

Notch signaling is the eminently conserved pathway of connection amidst neighboring cells and regulating cell proliferation and fate[23,24]. In some cancers, especially human T cell acute lymphoblastic leukemia, the activated mutation in NOTCH1 is seen[24]. NOTCH1 may act as a tumor suppressor as inactivated mutations in NOTCH1 are seen in 11-19%of cases having Oral squamous cell carcinoma[25,26[.

NOTCH1 is expressed in the basal cells of the oral squamous epithelium. It is important to note that the NOTCH1 expression is inhibited in oral epithelial dysplasia as well as in oral cancer[27]. Pickering had observed that 9% of patients having oral squamous cell carcinoma suppresses inactivating mutations in NOTCH1.In vitro proliferation of oral squamous cell carcinoma was inhibited by functional NOTCH1 signaling. NOTCH1 mutations were infrequent in Singaporean patients having oral tongue squamous cell carcinoma, whereas increased rates of NOTCH1 mutation is seen in Chinese patient having oral squamous cell carcinoma which advocates that NOTCH1 inactivation have composite genetic interactions in the occurrence of oral squamous cell carcinoma[28,29].

In oral squamous cell carcinoma, overexpression of Nrf2 was seen to develop a cancer phenotype. Hence, tumorigenesis may emerge from numerous forms of dysregulation of the Notch signaling pathway that suggest its concerned regulation is important for proper cell function[30].

Wnt/β-catenin:

The signaling pathway in Wnt/β-catenin is a conserved pathway that coordinates cell fate determination, differentiation, and proliferation. Upregulation of signaling pathways leads to oncogenesis in oral squamous cell

carcinoma. β-catenin is encoded by CTNNB1. It is found that mutations in CTNNB1 are rare in oral squamous cell carcinoma[26].

Epidermal growth factor receptor:

The epidermal growth factor receptor is a tyrosine kinase receptor. When activated, it upregulates considerable downstream signaling pathways that include AKT, MAPK, Jak/STAT, and ERK which are necessary for tumor growth, apoptosis, angiogenesis, proliferation, survival, invasion, and metastasis[31,32,33]. In 90% of cases of oral squamous cell carcinoma, EGFR levels are raised up[34]. Poor survival of patients and tumor aggressiveness are seen in the high expression of EGFR[35].

PI3K/AKT/mTOR:

The PI3K/AKT/mTOR pathway is a well-expressed signaling axis that is involved in the growth, survival, proliferation, and drug resistance of different cancer types[36,37]. In this pathway, activation of the PI3 family of protein kinase leads to PIP3 formation. This formation, in turn, activate AKT which further results in the activation of the different downstream target along with mTOR (mammalian target of rapamycin)[38]. Some studies have demonstrated that there is a direct role of activation of AKT signaling in oral squamous cell carcinoma. In immunohistochemistry analysis, staining of AKT, pmTOR, and pAKT are seen in considerable count in oral squamous cell carcinoma[39].

Via activation of AKT signaling, numerous tumor-associated pathways are shown to promote survival, invasion, growth, or drug resistance in oral squamous cell carcinoma[40,41]. Recently identified contributors which activates AKT signaling are FoxM1, RACK1, ZNF703, Nox1, Muc1, PDGF-D, CCL18[42,43,44].

p33ING1b gene:

ING tumor-suppressor proteins(ING 1-5), which is a growth inhibitor protein, have been discovered in past decades. It was suggested that ING1 was a negative growth regulator which acts as a class II tumor suppressor. It directly play role in apoptosis, oncogenesis, cell cycle regulation, and DNA repair. The p33ING1b protein is a widely expressed isoform of ING1 in human normal tissues. The ING1 proteins were intermittently downregulated.ING1 proteins were elevated in papillary thyroid carcinoma, melanoma, and ductal breast carcinoma[45,46]. ING1 was less periodically mutated in various human malignancies, such as colon carcinoma, brain tumor, oral squamous cell carcinoma, gastric tumor, and neuroblastomas[47,48].ING1 gene is located on human chromosome 13q33-34. It is observed that this region is involved in the advancement of various tumors[49,50].

Cylindromatosis (CYLD) gene:

CYLD (cylindromatosis) gene is a known tumor suppressor gene[51]. It inhibits the activation of NF-kB. The NF-kB plays important role in tumorigenesis, immune response, inflammation, and protection against apoptosis[52,53,54].

Neural cell adhesion molecule (NCAM)

NCAM or neural cell adhesion molecule is a family of cell surface glycoproteins.NCAM plays a key role in nervous system development, axonal outgrowth, fasciculation, regulating cell migration, and branching[55,56].The expression of NCAM is upregulated by TGF-b (transforming growth factor)[57]. It has been observed that NCAM expression is seen in numerous human neoplasms. Also, NCAM is participating in perineural invasions in numerous neoplasms such as gallbladder cancer, bile duct cancer, adenoid cystic carcinoma[58,59,60]. NCAM mediates the cell's adhesion through a Ca2+-independent hemophilic (NCAM—NCAM) binding mechanism. Also, NCAM mediates the adhesion between neurons and extracellular matrix through heterophilic binding[55].

Receptor-binding cancer antigen expressed on SiSo cells (RCAS1):

RCAS1 is known as type II membrane protein which is isolated as a human tumor-associated antigen.RCAS1 act as a ligand for a receptor that is present on immune cells such as B, T, and NK cells. The growth of receptor-expressing cells is inhibited by RCAS1[61,62]. It is observed that RCAS1 was regularly expressed in oral squamous cell carcinoma[63].

Tumor necrosis factor-related apoptosis inducing ligand (TRAIL):

TRAIL is also known as APO2 ligand or APO2L. It is a unique member of the TNF cytokine family.TRAIL has the capability to induce apoptosis. Few receptors help in the binding of TRAIL[64,65].

These are death receptor-4 (DR4),DR/5KILLER,decoy receptor-1(DcR1),DcR2. Death receptor-4 (DR4), as well as DR5/KILLER, consist of signal apoptosis and cytoplasmic death domains.DcR2 does not transducer death signal. DcR1

does not have a cytoplasmic tail and prohibits TRAIL function[66,67].

Interleukin (IL)-12 and IL-23:

The relationship between innate immunity, chronic inflammation, and cancer is now universally accepted. Various cancers originate at the chronic inflammation location[68,69]. It is observed that persistent use of anti-inflammatory drugs lessens the frequency of human tumors[70]. IL-23 has been recognized as an important factor that links tumor-associated inflammation and a loss of tumor immune surveillance.IL-23 consists of a p19 subunit which is associated with the IL-12p40 subunit.IL-12 is an association of IL-12p35 and IL-12p40 subunit[71]. The various signal-transduction components used by IL-23 include Tyk2, Janus kinase2, and Signal transducer as well as an activator of transcription Stat1, Stat3, Stat4, and Stat5[72]. In some studies, it has been observed that IL-12 has dominant antitumor activity in various murine tumor models which leads to regression of established tumors. Also, it inhibits the development of experimental and spontaneous metastasis[73,74].

Nuclear factor (NF)-kappaB:

Nuclear factor-kB (NF-kB) plays important role in immune response, tumorigenesis, inflammation and atability against apoptosis[75,76,77].

Hypoxia inducible factor (HIF)-1alpha:

Hypoxia inducible factor (HIF)-1alpha plays a pivotal role in biological processes specifically carcinogenesis and angiogenesis[78,79]. HIF-1a upregulates various essential factors for tumor expansion which includes VEGF. In a few cancers, tumor aggressiveness is seen to be associated with overexpression of HIF-1a[80,81,82].

Epigenetics:

Epigenetic alterations to the genome bring one of the considerable mechanisms for gene expression regulation. Various processes like methylation and demethylation of gene promoter province play an important role in gene silencing or expression of the gene. This gene silencing as a result of methylation is one of the hallmarks of carcinogenesis[83,84].

It is observed that many times p16 is inactivated because of promoter hypermethylation in oral squamous cell carcinoma[85,86,87]. The methylation of the p16 promoter occurs due to upregulated expression of DNA methyltransferases as a consequence of regular use of tobacco[88,89]. A large number of alternative genes are also hypermethylated in promoter regions in oral squamous cell carcinoma which includes RASSF1A, RASSF2A, CDH1, p14,p15, MLH1, and DAPK. These genes play a crucial role in different cancer-promoting signaling networks like Wnt signaling, DNA repair, Cell adhesion, and Cell cycle arrest and apoptosis[90,91,92,93].

Metastasis/invasion:

Metastasis is known as a complicated process in which selected malignant cells acquire the potential to remain alive in a distant environment. The main steps in metastasis include angiogenesis and epithelial-mesenchymal transition. Angiogenesis is a process that concentrates around the activation of VEGF (vascular endothelial growth factor).VEGFA is a useful prognostic indicator for oral tongue squamous cell carcinoma. Also, overexpression of VEGFA shows poor survival[94,95].

Epithelial-mesenchymal transition characteristically involves the reduction of molecules that influence cell adhesion such as upregulation of cytoskeletal protein (Vimentin) and upregulation of mesenchymal protein (N-cadherin).

In various immunohistochemical studies, oral cancers show increased expression of Vimentin and decreased expression of E-cadherin. Hence, it recommends that cancers take advantage of epithelial-mesenchymal transition for invading and metastasizing[96,97]. Epithelial-mesenchymal transition is also eminently driven by the expression of TGF-β i.e. transforming growth factor. TGF-β activates cell spreading and also causes partition of cell borders. It has been observed that overexpression of TGF-β1 causes migration and invasiveness of oral squamous cell carcinoma and advances the malignancy[98,99]. Phosphorylation of β-catenin results in dissociation from the complex with E-cadherin, which leads to loss of cell adhesion and consequential invasion of tumor cell[100,101].

Conclusion:

Our knowledge of molecular basis of oral squamous cell carcinoma grows promptly in past years. Various genetic proceeding that conclude in carcinogenesis comprises of oncogenes activation and deactivation of tumor suppressor genes.Recent progress in microarray technologies and DNA

sequencing provides a exceptional favourable circumstances for thousand of genes to be managed at the same time.

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