REVIEW

Cytogenetics and oral carcinoma

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ABSTRACT

Oral carcinoma has been associated with the use of tobacco, alcoholism, cigarette smoking, and chronic trauma as the main etiological agents. Undoubtedly, these factors play an important role in oral carcinogenesis but they may not solely account for cancer initiation, development, and progression. Cytogenetics provides important insights into the molecular basis of the etiopathogenesis of oral squamous cell carcinoma.

Carcinogenesis occurs as a result of interactions between host immunity, oncogenic triggers, microbiomes, viruses, and regulatory gene responses. The mutagenic factors cause irreversible damage to tissue cells, leading to genetic mutations in pre-invasive cell populations, resulting in their malignant transformations.

There is a major paradigm shift in our understanding from traditional beliefs towards contribution of polymorphic genetic transformations in oral cancer development. The present review will try to focus on causal effects of gene polymorphisms in development of oral cancers and evaluate its critical importance in disease prevention as well as progression.

KEYWORDS: Oral squamous cell carcinoma, microbiomes, oncogenes

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INTRODUCTION

Oncogenesis is a complex process involving multiple channels, leading to quantitative alteration of genetic material within the signal transduction pathways, responsible for maintaining normal cellular physiology. (Vogelstein and Kinzler, 1993)

Cancer registries provide staggering veritable statistics of cancer globally. According to the World Health Organisation, approximately 657,000 new cases of oropharyngeal cancers are reported every year accounting for>330,000 deaths. ("WHO | Oral Cancer" n.d.) The highest incidence of oral cancer is observed in the Indian subcontinent, Sri Lanka, and Taiwan, owing to the high rate of cigarette smoking and use of smokeless tobacco/ areca nuts in these areas. (Warnakulasuriya 2010)

Not all oral cancers can be attributed to the known etiologies of smoke and smokeless tobacco. (Conway, Purkayastha, and Chestnutt 2018) The role of other etiologies like human papillomavirus, genetics, low socioeconomic status, anemia, and periodontal/oral health is being researched as probable triggers.

Through the years the evidence is accumulating regarding the role of multiple genetic events (approximately 6-10) in the development of single or multiple site oral cancers. This review intends to shed light on the interspersing of cytogenetics in the fine framework of cellular genesis, differentiation, and proliferation, shifting the balance from normal physiologic function towards malignant transformation.

Cancer Hallmarks

Six hallmarks (Figure1) of molecular pathways are: (Hanahan and Weinberg 2000)

- Oncogenes: acquisition of growth signaling autonomy
- Tumour suppressor genes: growth-inhibitory signal pathways
- Evasion of apoptosis
- Cellular immortalization
- Angiogenesis
- Invasion and metastasis

New hallmarks to these events were added later: (Fouad and Aanei 2017)

- Reprogramming of energy metabolism
- Evasion of the immune response

Fouad & Aanei (2017) also described enabling traits towards carcinogenesis as genome instability and mutation, and inflammation-promoting tumorigenesis.

Analysis of tumor DNA

Various methods are used in reported literature for analyzing tumor DNA. Commonly used methods are in situ hybridization, automatic DNA sequencing, microarrays, Southern/Northern/ Western blots, Polymerase chain reaction (PCR). In situ hybridization is used to reveal the location of specific nucleic acid/ gene sequence of DNA/RNA in cells, preserved tissue sections, or entire tissue.

Human DNA is composed of four nucleotide bases: adenine, guanine, thymine, and cytosine. Automatic DNA sequencer analyses any abnormality in the sequence of these nucleotides. Northern blotting is used for RNA detection, southern blotting for DNA, and Western blotting for protein detection to check for aggregation of any respective abnormal gene.

Microarrays are laboratory tests to detect the expression of thousands of genes at a single time point. They are useful in screening of differential gene expressions. Results of microarrays require validation through other methods like Southern blotting or quantitative real-time PCR. Real-time PCR is the current gold standard in validating gene expressions. (Rajeevan et al. 2001) Studies related to oral cancer have frequently utilized microarrays with quantitative PCR.

Omics technology in Cancer pathophysiology

In today's era of advanced technology, the knowledge paradigm in cancer pathobiology has

shifted from morphological features towards omics-based systems. OSCC is a complex genetic disease characterized by tumor heterogeneity and plasticity, (Stucky et al. 2017) and therefore, omics technology (genomics, transcriptomics, proteomics, and metabolomics) is utilized to identify biomarkers in tissue biopsies, circulating onco-cells, and body fluids (saliva, gingival crevicular fluid, etc.).

Familial aggregation

Familial aggregation is seen with oral cancers that begin at an early age. Risk is associated with immediate family members as well as towards extended family. (Ankathil et al. 1996; Jefferies et al. 1999) Studies have reported an increased risk for multiple primary tumors in families with more than one patient diagnosed with oral squamous cell carcinoma. (Copper et al. 1995; Foulkes et al. 1995) Familial aggregation is a strong indicator of genetic mutations resulting in oral carcinoma, which are hereditarily transmitted in families.

Genetic alterations

Gene alterations occur as point mutations of single nuclear base pair (SNP), amplifications/ rearrangements, and/or deletions of base pairs.

Point mutations may either cause overactivity or inactivity of genetic response. This kind of gene alteration is observed in K-ras and p-53 gene.

Evidence reports malignant neoplasms to be associated with amplification or rearrangement of genes affecting excitatory or inhibitory pathways, respectively. (Todd, Donoff, and Wong 1997) Multiple chromosomal regions (3q, 5p, 7p, 8q, 9p, 10p, 11q) are found to be frequently amplified. Similarly, chromosomal regions 3p and 8p are found to be frequently deleted. Co-alteration of 7p, 8q, 9p, and 11q are associated with the change in clinical and pathologic presentation of oral squamous cell carcinoma (OSCC) resulting in poor survival. These regions are found to contain genes playing pivotal roles in tumor genesis pathways. (Vincent-Chong et al. 2017)

Deletion in chromosome area 9p21-22 has been observed in more than 2/3rds of oral carcinomas. (Ah-See et al. 1994; Nawroz et al. 1994) This area is, therefore, a strong predictor for oral carcinogenesis.

Oral carcinogenesis is a complex process involving multi-step genetic events involving quantitative/qualitative alteration of signal transduction pathways governing normal physiologic functions.

These pathways govern cell biology of the oral epithelium through cellular division, differentiation, and cell-senescence. These excitatory and inhibitory pathways are affected through gene alteration making the oral epithelial cell functionally independent and distinct from normal neighboring cells. These cells start rapid replication, sequester blood vessels to fulfill nutritional demands of the growing mass, cause signal deletion or amplification for abnormal functional changes, and slowly start invading neighboring normal tissue. (Todd, Donoff, and Wong 1997)

Alteration in the normal function of oncogenes and tumor-suppressor genes induce neoplasia. (Sugerman, Joseph, and Savage 1995) The genetic damage of the dominant type causes disruption of oncogene/proto-oncogene function leading to gain of function in cells, and recessive types cause changes in tumor-suppressor genes or anti-oncogenes leading to loss of function. (Bishop 1991).

Oncogenes

Oncogenes are defined as altered regulatory genes that promote uncontrolled growth through signaling cell transduction pathways. (Bishop 1991) Mutation in these genes cause overproduction of excitatory proteins or increase the function of proteins.

These genes are initiators of the cancer process and do not cause epithelial transformation per se. Single gene mutation leads to cellular changes

responsible for tumor formation. Various oncogenes are responsible for oral cancer initiation and progression. ("Oral Cancer | Cancer Genetics Web" n.d.) (Table1)

Oncogenes are characterized by the function of proto-oncogenes (normal counterparts) that regulate normal physiologic biochemical pathways of growth and differentiation in cells and tissues. They can be classified as:

- Growth factors (GF): transforming GF, fibroblast GF, platelet-derived GF
- Cell surface receptors: epidermal GF receptor, fibroblast GF receptor
- Intracellular signal transduction pathways: RAS genes (Hras, Kras, and Nras)
- DNA binding nuclear proteins transcription factors: c-Myc, c-Fos, and c-Jun
- Cell cycle proteins: cyclins and cyclin-dependent protein kinases
- Apoptosis inhibitors: BCL-2

The most commonly associated oncogenes with oral carcinogenesis are EGFR/c-erb1, members of the ras gene family, Bcl-1, PRAD-1, c-myc, int-2, and hst-1. (Todd, Donoff, and Wong 1997)

Table 1: Oral Cancer and related genes.

Gene	Location
ABCG2	4q22.1
ADH1C	4q23
AIDA	1q41
ALDH2	12q24.12
ANXA8	10q11.22
BAX	19q13.33
BCL2	18q21.33
BCL2A1	15q25.1
BDNF	11p14.1
CA9	9p13.3
CDK2AP1	12q24.31
CFLAR	2q33-q34
CSMD1	8p23.2
CTTN	11q13.3
DDR2	1q23.3
DEC1	9q33.1
EDNRB	13q22.3
ENDOU	12q13.1
FAT1	4q35.2
FGF19	11q13.3
FGF4	11q13.3
GHRH	20q11.23
GSTM1	1p13.3
GSTM3	1p13.3
GSTT1	22q11.23

HOXC13	12q13.13
IL6	7p15.3
IMP3	15q24.2
ING5	2q37.3
ITGB4	17q25.1
KIAA1524	3q13.13
LAMC2	1q25.3
MALL	2q13
MARCO	2q14.2
MIRLET7I	12q14.1
MMP10	11q22.2
MMP2	16q12.2
NAT2	8p22
NODAL	10q22.1
NTRK2	9q21.33
PDPN	1p36.21
PRRX1	1q24.2
RPS6	9p22.1
RXRB	6p21.3
S100A7	1q21.3
SERPINE1	7q22.1
SLPI	20q13.12
SPRR2B	1q21.3
THBS2	6q27
TMC6	17q25.3
TMC8	17q25.3
TNFRSF8	1p36.22
TWIST1	7p21.1
WISP1	8q24.22
WNT11	11q13.5

Source: ("Oral Cancer | Cancer Genetics Web" n.d.)

Tumour suppressor genes

Tumour suppressor genes are those genes that encode negative signal transduction pathways proteins modulating excitatory pathways, and suppressing their function resulting in cells to function under internal/external stresses. (Rivlin et al. 2011) These are also known as anti-oncogenes or recessive oncogenes. Cellular changes resulting in the transformation of the pre-malignant cell to malignant cell is due to the inactivation of tumor-suppressor genes.

Inactivation of these genes occurs due to point mutations, deletions, rearrangements, or hyper-methylation of both gene copies. Tumor-suppressor genes may be an inherited trait leading to early identification through pediatric tumors.

The most commonly studied tumor-suppressor gene is p53. This gene suppresses cell division at the G1-S phase of the cell cycle, induces DNA repair, and causes natural physiologic cell-death. This gene mutation is linked to tobacco use and

smoking-induced oral carcinoma. (Hooper 1994; Spandidos, Lamothe, and Field 1985)

The mechanism by which tumor p53 gene exerts its role in cancer progression:

- Mutant p53 acquires gain-of-function (GOF) property which initiates cellular migration, invasion, and metastasis.
- Tumour metastasis occurs through epithelial-to-mesenchymal transition (EMT). Mutant p53 promotes EMT manifesting as loss of cell-cell adhesion, and increased cell mortality through key transcriptional regulators (TWIST1 and SLUG).
- P53 inhibits the transcriptional activity of TAp63α (Tp63), which augments cell invasion.

Field cancerization

OSCC progresses from a pre-malignant lesion (erythroplakia, leukoplakia) to malignant carcinoma. The concept of field cancerization was given by Slaughter et al. (1953) This refers to a large, pre-malignant field of carcinogen exposed epithelium with no apparent clinical or histopathological changes. The changes that occur are at the molecular level. The genetic component of cells gets altered through the accumulation of genetic and epigenetic changes, resulting in cell-cycle dysregulation. This further causes uncontrolled cell proliferation leading to malignant transformation. (Feller et al. 2013)
OSCC premalignant field is characterized by the presence of mutated tumor suppressor gene p53. (Braakhuis et al. 2003) The tumor p53 cells form a patch, which gradually expands through additional mutations causing cell proliferation and eventually displacement of normal epithelium. (van Houten et al. 2002)

Cancer initiating cells

OSCC shows tumor heterogeneity and initiates with a specialized population of cancer-initiating cells (CIC). This cell possesses stemness i.e., the ability of self-renewal and develops heterogeneous neoplastic cell clones. The various hypothesis is proposed to explain the origin of CICs but further research is required to clarify the origin. CICs have a slow cell cycle and are resistant to high-proliferative cell targeting chemotherapeutics. (Bjerkvig et al. 2005; Prince et al. 2007) These cells regenerate following chemotherapy and cause recurrence.
Putative CICs for OSCC are characterized by cell surface markers like CD133 and CD44. B-cell specific Moloney murine leukemia virus insertion site 1 (Bmi1) is critical in cell senescence and linked through recent evidence to OSCC invasiveness and regional metastasis. (Chen et al. 2017; Tanaka et al. 2016)

Multistep- cancer progression

Multistep progression of OSCC requires altered genetic and epigenetic accumulations in oncogenes and tumor-suppressor genes. Interactions between cancer cells and the micro-environment increase progressiveness and invasiveness of the tumor.
Chromosomal instability through telomerase dysfunction or loss of heterozygosity (LOH) has been researched extensively. (Haddad and Shin 2008)

Mutations of telomerase reverse transcriptase (TERT) have been seen in >80% of OSCC. (Leemans, Braakhuis, and Brakenhoff 2011) LOH of chromosome 3p, 8p, 9p (p16), and 17p (p53) are associated with OSCC initiation and progression. (Leemans, Braakhuis, and Brakenhoff 2011) LOH at 17p13 (p53) locus is associated with higher evidence of OSCC.

Besides, mutations of CDKN2A, CCND1, PIK3CA, PTEN, and HRAS can also cause OSCC initiation and progression. (Agrawal et al. 2011; Stadler et al. 2008; Stransky et al. 2011)

DNA methylation and histone modifications are epigenetic alterations that regulate gene expression through adjustment of chromosomal structures. Transcriptional silencing of the tumor

suppressor gene (p16) is detected in the majority of OSCC tumors. (Gasche and Goel 2012; Jithesh et al. 2013; Kulkarni and Saranath 2004) Histones are structural proteins packed with DNA structure and their modification through acetylation or methylation dysregulate transcriptional activities.

MicroRNAs

MicroRNAs (miRNAs) also play a significant role in oncogenesis. They alter the expression of the tumor-suppressor gene or oncogenes. Their significance in cancer initiation and progression has made them a robust prognostic predictor of OSCC. (Lu et al. 2005) characterization of OSCC based on 61 miRNAs has been shown to have 93% accuracy in distinguishing normal oral epithelial cells from malignant counterparts. (Lajer et al. 2011)

miRNAs are 18–23 nucleotide-long, single-stranded, noncoding RNAs. Their function is post-transcriptional repression of their target genes. Their expression is altered through transcriptional dysregulation, epigenetic modifications, chromosomal changes, SNPs, and processing defects. Altered miRNAs linked to OSCC are downregulated miR-375. (Koshizuka et al. 2017)

CONCLUSION

OSCC is an invasive, highly proliferative, heterogeneous, and distinct type of head and neck cancer with known etiologies like tobacco, alcohol, and areca-nut. Not all patients with these known etiologies develop oral cancer. The underlying genetic mechanism plays a critical role in cancer initiation, progression, and metastasis. Recent advances in omics technologies have made our understanding of the disease better and provide scope for the development of biomarkers for early diagnosis and personalized precision medicine. Familial aggregation of disease provides indisputable evidence towards the role of genetics in OSCC. Evidence suggests these cancers to be predominantly HPV negative with the presence of TP53 loss-of-function mutations.

The prognosis for OSCC remains dismal (approximately 50% 5-year survival rate) for advanced OSCC. These cancers have genetic and epigenetic aberrations, with a wide range of cancer-initiating pathways (eg, PI3KPTEN- AKT-mTOR).

Investigation of new and currently identified biomarkers, oncogenes, tumor suppressor pathways through validated cohorts, or multi-center clinical trials may increase the quality of life and survival rates through the development of personalized therapy for OSCC patients.

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Declaration of originality

The authors declare that they have not copied text, figure or data from a particular source without appropriately citing it.

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