Review Article

DOI: 10.7860/JCDR/2013/5552.3281

Dentistry Section

Early Detection of Oral Squamous Cell Carcinoma (OSCC) – Role of Genetics: A Literature Review

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Key words: Premalignancy, Potentially malignant, Genetics, Oral squamous cell carcinoma

According to GLOBOCAN 2008, there were 12.7 million new cancer cases and 7.6 million deaths. Among these, 56% of the cases and 64% of the deaths occurred in the economically developing world. Oral cancer accounted for 3-4% of all cancers. There were 263,900 oral cancer cases, with almost two-thirds of them occurring in men. Oral cancer is among the leading cancer types in south central Asian men. In India, oral cancer is the leading cancer type among men and third most common cancer among women [1].

There have also been impressive advances in recent years regarding the detection, prevention, and treatment of OSCC. Unfortunately, however, the overall 5-year survival for OSCC continues to be modest at its best. OSCC survival is highly dependent on the stage of the tumour at diagnosis. For example, Stage I cancers have an 80% 5-year survival rate, while the survival rate decreases to 20% for Stage IV lesions [2]. To improve long-term outcomes, an early detection, in conjunction with primary and secondary prevention strategies, is critical.

Screening and an early detection are believed to decrease both the morbidity and mortality which are associated with OSCC, because unlike many anatomic sites, in the oral cavity, pre-malignant lesions are often visible on clinical examination. However, an accurate discrimination between premalignant vs reactive/inflammatory lesions via conventional visual and tactile examinations alone is problematic. As the malignant potential of oral lesions cannot be accurately predicted solely on the basis of their clinical characteristics, a histological evaluation is essential for all suspicious lesions. The definition of an oral mucosal pre-malignancy that is based on a conventional histologic examination can also be problematic. Lesions are currently considered as pre-cancerous when there are cytomorphologic changes which are consistent with dysplasia. However, the various criteria for diagnosing and grading dysplasia are controversial, highly subjective and open to a wide range of interpretation, even among highly qualified pathologists [3,4]. In addition, no definitive criteria currently exist for predicting the risk of a cancerous transformation of individual dysplastic lesions; even dysplastic oral lesions have been reported to undergo spontaneous regression.

Therefore, conventional histologic findings can only be utilized to indicate that a given lesion may have a malignant potential, and that it cannot be used for the prediction of a malignant change. Hence, two key issues should be considered:

1. In general, a progression to OSCC may not occur in a linear fashion over a uniform period of time. Rather, there are subsets of lesions with histologic evidences of dysplasia, that may or may not progress to OSCC.
2. Similarly, the histologically normal' appearing mucosal lesions may truly be benign or they may represent molecular premalignant lesions that have not yet developed morphologic /cytologic changes which are consistent with dysplasia [5].

Current modeling postulates that the development of cancer is driven by the accumulation of genetic and epigenetic changes within a clonal population of cells. These genotypic alterations can affect hundreds of genes, leading to phenotypic changes in critical cellular functions, such as resistance to cell death, increased proliferation, induction of angiogenesis, and the ability to invade and metastasize. The mechanisms which underlie these genetic and epigenetic aberrations include, but are not limited to, genomic instability through chromosomal rearrangements, amplifications, deletions, methylations and mutations. This article gives a brief review on various genetic and epigenetic alterations which are observed in the potentially malignant lesions that are likely to progress to cancer.

GENETIC AND EPIGENETIC CHANGES IN POTENTIALLY MALIGNANT AND MALIGNANT ORAL LESIONS

1. Aneuploidy

Chromosomal instability often leads to an imbalanced DNA content and the generation of near-diploid or aneuploid clones. Aneuploidy may result from a gene dose imbalance, loss of TSGs, gain of tumour promoting genes or oncogenes, or formation of fusion genes that leads to an increased survival and proliferation advantage. Approximately50–60% of oral cancers are aneuploid, with one study reporting a figure of 90% [6, 7, 8]. Aneuploidy in OSCC has also been shown to be associated with higher incidences of local recurrence and lymph node metastases.

2. miRNA

The discovery of microRNA (miRNA), 20–22 nucleotide-long members of the non-coding RNA family, adds another layer of gene regulation that is altered as cancer develops. They may be present as intergenic transcription units or they may be found in the intronic sequences of protein-coding genes. More than 1000s of these sequences have been identified and functional studies have identified that miRNAs act as conventional tumour suppressors or as oncogenes, and affect the translation or stability of target mRNA. Most of them are negative regulators of gene expression and have fundamental roles in biologic processes, with this function being dysregulated as cancer develops.

3. Loss of Heterozygosity (LOH) and Microsatellite Instability or Allelic Imbalance (AI)

Loss of heterozygosity and AI has been relevant targets in cancer research. An AI may occur when one copy of a polymorphic marker (with two slightly different alleles) is lost (LOH) or amplified (allelic gain). The term, 'LOH' is commonly used to describe this process, but as allelic gains occur very frequently, and as they may be more common, an AI describes the process more accurately. AIs occur at loci across the genome at low frequencies and at higher frequencies at 3p (3p24–25, 3p21, 3p13–14), 9p21 (p16),17p13 (p53) and

8p22–24, with the loci at 13q14, 18q and 21q being implicated in some studies. A consensus has emerged that the AIs at 3p and 9p provide useful evidence on the accumulation of genetic damages in potentially malignant lesions.

4. Epigenetic events

Epigenetic changes involve modifications of DNA and histones that are not coded in the DNA sequence, although these changes are heritable [9].Three systems are involved: DNA hypermethylation, RNA-associated post-transcriptional silencing, and histone modification. Of these, DNA methylation has been studied in OSCC. In normal tissues, unmethylated cytosine is found in high densities in CpG islands (areas with high concentrations of cytosine and guanine) that map close to a promoter region in 40% of mammalian genes [9]. This unmethylated state is associated with a high rate of transcriptional activity. Hypermethylation of TSGs, which is mediated through the enzyme, DNA methyl transferase, results in a stable transcriptional silencing of tumour suppressor activity. This process has been detected in oral OSCC and it is a hallmark of many other cancers as well. In OSCC, hypermethylation of p16 occurs in 50–73% of the cases and that of p15 occurs in 60% of the cases [10-13]. Hypermethylations of p14ARK, p16 INK4a, P15, MGMT, DAPK, GSTP1 and RARB have been seen in dysplasias and in histologically normal appearing margins of OSCC resections.

5. Telomerase Regulation

Telomeres are specialized areas of the distal ends of chromosomes, which are composed of chromatin, which are formed through tandem repeats of the sequence TTAGGG, which are bound to specific telomere-binding proteins. They are progressively shortened with each cell division, ultimately resulting in aging and senescence of cells. As telomere loss limits lifespan of cells, the loss also reduces the probability of cancer development. Telomerase is an enzyme that directs the synthesis and maintenance of these telomeres and it is composed of hTR (human telomerase RNA, the RNA template), hTEP1 or TP1(telomerase-associated protein 1) and hTERT (human telomerase reverse transcriptase) [14]. Cancer cells are able to stabilize their telomeres by activating telomerase, thereby bypassing senescence and facilitating cell immortalization [15].

6. Proliferation Markers

It has been generally accepted that increased cell proliferation is associated with the progression in the multi-step process of carcinogenesis. The immunohistochemical methods which are used for detecting proliferation markers, such as proliferating cell nuclear antigen (PCNA), mini chromosome-maintenance protein 2 (MCM2) and Ki-67, have been widely used as possible indicators of genetic abnormalities, which are typical of a malignant progression. The Ki-67 antigen is one of the best known proliferation markers, as its expression is seen in proliferating cells (G1, S and G2 phases), but not in resting cells (G0 phase). MCM2 is expressed throughout the cell cycle, including the cells which leave G0 to enter into the early G1 phase, distinguishing them from Ki67. PCNA, another marker which is frequently used as a measure of the proliferation, is an essential factor, both for replication and repair of DNA.

7. The p53 Family: p53, p63, p73, p21 and p27

i. p53 is a TSG which is located on chromosome 17p13. p53 plays a major role in cell-cycle progression, cellular differentiation, DNA repair and apoptosis, and it is regarded as a guardian of the genome. Loss of p53 function diminishes the regulation of cell cycle arrest and apoptosis, thereby altering the ability of cells to respond to stress or damage (such as DNA damage, hypoxia, and oncogene activation). This can subsequently lead to genomic instability and the accumulation of additional genetic alterations. p53 is the most commonly inactivated TSG in human cancer, including OSCC [16]. Various genetic events can lead to inactivation of p53, which include mutation, inactivation through interaction with a viral protein of the 'oncogenic' HPV subtype, (such as HPV16 or HPV18), or through loss of one allele as a result of LOH [17-19]. Immunohistochemical (IHC) expression of a mutant p53 protein has been correlated with an increased risk for secondary tumours, an early recurrence, metastatic spread, and resistance to chemotherapy or radiation therapy [20-22]. Poeta et al., in 2007, reported that inactivation of p53 in OSCC was associated with a reduced survival after a surgical treatment [23].

ii. p63 and p73 are members of the p53 family, and they are related both structurally and functionally to p53 [24] (Smeenk and Lohrum, 2010). Both cooperate with p53 to induce apoptosis, thus suggesting that they have a role in the regulation of DNA damage-induced cell death.

iii. The CDK inhibitor (CDKI) p21 mediates growth arrest, following a DNA damage, by inactivating members of the cyclin family. An altered expression of p21 in OSCC has been reported with respect to its correlation with tumour biology and clinical outcomes [25-28].

iv. The p27 gene which is located on chromosome shares sequence homology with p21, and it acts as a p53 independent, negative cell cycle regulator which is involved in G1 arrest. It associates with several CDK complexes, resulting in loss of their activity and inability in phosphorylating the retinoblastoma (Rb) gene. Although it rarely gets mutated, reduced levels of p27 protein expression have been reported in various human tumours, including OSCC [29].

8. The Rb Family: Rb, p16

The Rb gene was the first TSG which was identified and it plays a key role in the regulation of cellular proliferation. There is compelling evidence that several components of the Rb pathway are altered in human cancer, including OSCC [30]. The expression of Rb protein in OSCC has been variably reported. Many studies have reported loss of Rb protein expression, while others have shown elevated levels of its expression [31-35]. Inactivation of the p16 TSG is a common event in various types of cancer and it may be one of the first TSGs to be inactivated in OSCC. Inactivation of this gene may have a prognostic relevance [32,33,35-40]. P16 is a member of a family of negative cell cycle regulatory proteins, that inhibits the activity of cyclin D–CDK4⁄6 complexes, thereby preventing Rb phosphorylation.

9. Receptor Tyrosine Kinase Pathways

Several signal transduction pathways are frequently altered in cancer and they share common nodes and interact as a network. Their modification can affect cell survival, cell proliferation, morphology, and angiogenesis. Comprehension of the underlying pathways which govern the progression of oral premalignant lesions is thus of utmost importance. A number of growth factors, which include Platelet-Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), Nerve Growth Factor (NGF), and Transforming Growth Factor-a (TGF-a) family members, signal by inducing dimerization and activation of receptors that are protein tyrosine kinases. Through the tyrosine kinase cascade, the receptor tyrosine kinase has many downstream signaling targets that are associated with carcinogenesis [5].

CONCLUSION

The potential to identify accurately and prospectively the subset of dysplastic lesions which are likely to progress through dysplasia to cancer are of premier scientific and clinical significance. Analysis of correlations between biomarkers, stages of dysplasia and their

progression to OSCC is complex and it requires incorporation of multiple variables. Biomarker profiles are of high value in this regards and they may ultimately supercede histopathologic staging in the future.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jan 04, 2013**
Date of Peer Review: **Mar 18, 2013**
Date of Acceptance: **Mar 28, 2013**
Date of Publishing: **Aug 01, 2013**