Promoter hypermethylation of Tumor Suppressor Genes in Oral Squamous Cell Carcinoma

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ABSTRACT

The oral squamous cell carcinoma (OSCC) is the common cancer subtype in the world causing millions of deaths. The detection and analysis of these oral lesions are done through traditional biopsy detection method, which is painful and time taking. The major reason for the oral cancer were found to be smoking and tobacco chewing despite of the several other reasons which are responsible for cancer. Epigenetic changes are one of those changes which occurs due to environment and dietary factors. These epigenetic changes are reversible and can be detected through MS-PCR. Various tumor suppressor genes get hypermethylated cytosine and are in turn inactivated. These changes leads to the downregulation or silencing of the gene which leads to cancer. These tumor suppressor genes may play vital role in the diagnosis of cancer and provide better alternative as a diagnostic biomarker.

KEYWORDS: Oral Squamous Cell Carcinoma, Hypermethylation, Epigenetic changes, Diagnostic.

INTRODUCTION

Cancer is a leading cause for morbidity and mortality worldwide. Approximately 14 million new case are arising yearly among worldwide, stating 8.2 million cancer related deaths in 2012 alone. Statistical studies have shown that among head and neck cancer oral squamous cell carcinoma (OSCC) is one of the most commonly occurring cancer\textsuperscript{1}. Among new cases of oral, laryngeal and pharyngeal cancers nearly 40,000 cases are expected in 2006, collectively causing 11,000 deaths\textsuperscript{2}. The fact that there are more than 100 different types of cancer, with most cancers having multiple possible causes; cancer has become a war that needs to be fought over. Squamous cell carcinoma which may affect any anatomical site in the mouth, commonly affects the tongue and the floor of the mouth and accounts for 90\% of all oral cancers\textsuperscript{3}.

Epidemiology

Oral squamous cell carcinoma that accounts for 3–5\% of all human malignancies is ranked sixth most common cancer in the world\textsuperscript{4}. The most frequent site of oral squamous cell carcinoma is the region of head and neck which encompasses tumors arising from the epithelium of the nasal, oral cavity, pharynx, and larynx\textsuperscript{5}. Annual incident of 50,000 cases of head and neck cancer has been noticed\textsuperscript{6}. The main risk factors involved as causative agent in OSCC are smoking and alcohol consumption. Revised investigations by Petti about prevalence and/or incidence of OSCC concludes that smoking is a major risk factor in ¼ of cases, between 7 to 19\% of cases are attributable to alcohol consumption and 10 to 15\% cases are caused due to micronutrient deficiency\textsuperscript{7}. Various case-control studies have been conducted in India to study the higher prevalence of OSCC in Indian subcontinent. In one such study on patients diagnosed with oral cancer (n=388), statistical parameters were studied including risk factors and an equal number of age and sex-matched controls to assess the effect of lifestyle factors (tobacco chewing, smoking, alcohol drinking, diet and dental care) on the risk of oral cancer. The results showed that use of tobacco (chewing) alone and alcohol drinking emerged as significant risk factors for oral cancer (odds ratio - OR=11.34). The global prevalence of oral potentially malignant disorders ranges approximately from 1 to 5\%\textsuperscript{8}. The first step in the prevention and control of OSCC is to increase awareness among the general public and policy makers to emphasize that these risk factors are modifiable\textsuperscript{9}.

Risk Factor

Oral Squamous Cell Carcinoma (OSCC) consists of one third of total cancer malignancies. The factors that increase the risk of developing
OSCC are unhygienic oral habits, dietary factors and addiction interests as of chewing tobacco, betel quid and areca-nut. It is the dose, frequency and duration of use which determine the adversity of mutagenic effects of these causative agents on normal cell. The incidence and prevalence of OSCC has been increasing on an alarming rate, particularly in younger persons. It has been reported that different carcinogens activate or inhibit specific pathways during cancer development and progression. In Indian population the most prevalent tumor sites are mouth and oropharynx. In Indian Subcontinent the prevalent oral habits of tobacco and betel quid chewing, bidi (tobacco flakes wrapped in a tendu leaf) smoking, cannabis and alcohol consumption have been documented as major risk factors for OSCC. The above forms of tobacco are known to contain hydrocarbons and several potent nitrosamines which are carcinogenic and act via initiation and promotion of oral cancer.

Several studies have been carried out to explain the role of viruses in the development of oral squamous cell carcinoma. Viruses which have been frequently studied are Human papilloma virus (HPV), Epstein-Barr virus (EBV) and Herpes Simplex virus type 1 (HSV-1). This analysis showed that HPV significantly increases the risk for OSCC, compared with controls (OR 3.98; 95% CI: 2.62–6.02). HPV proteins, especially the oncoproteins E6 and E7 of the high risk HPVs (HRHPVs), interact with different degrees of affinity with host cell proteins. HRHPVs disturb the normal epithelial differentiation and apoptosis by stimulating cellular proliferation, DNA synthesis and inhibition of cell cycle regulators.

**Epigenetic Changes**

The term epigenetics was first introduced by Waddington. Epigenetic changes had played a big role in cancer as they cause a loss in the functionality of the gene without any genetic alteration or mutation. Epigenetic changes denote reversible and heritable modifications in gene expression without any alterations in the DNA sequence. Epigenetic changes vary from population to population and are influenced by environmental and dietary factors that vary along with population and regional changes. The epigenetics changes occurs at 5th carbon of DNA base Cytosine nucleotide present in the DNA sequence. This change is marked by addition of the methyl group on the cytosine nucleotide which is carried out by DNA methyltransferases. These enzymes have very important role in the regulation of gene and along with it also provides prevention of gene from viral and other external radicals. Aberrant DNA methylation includes genome-wide hypomethylation as well as promoter CpG island hypermethylation. Several studies have reported certain cancer-related genes to be frequently methylated in oral malignancies.

**DNA Promoter Methylation**

Methylation can physically inhibit the transcription of the gene or methylation can lead to the recruitment of transcription factors that repress transcription, leading to the same end result - down regulation of gene expression that leads to decreased levels of the tumor suppressor protein. In this phenomenon the promoter region of the gene is hypermethylated causing methyl group to bind at the 5th position. Promoter hypermethylation does not occur as a separate event; instead the methyl group comes from global hypomethylation in the gene. Changes in the pattern of the epigenetic methylation of DNA promoter region leads to the occurrence of the cancer initiation.

Aberrant methylation of CpG islands is an important event associated with the silencing of certain tumor suppressor genes. These genes are central to the development of many solid tumours and their silencing may occur in the absence of genetic change. Restriction enzymes are one of the methods used for determination of promoter methylation. Other methods include genomic bisulphite sequencing and microarray-based methylation analysis, however, the chief method in use till date is methylation-specific PCR following bisulphite treatment (MSP). MSP is also potentially liable to oversensitivity following the high number of PCR cycles sometimes reported, for example 80 cycles.

**Genes found to be hypermethylated in OSCC**

Cancer is not caused by the action and involvement of a single gene. Research reveals that many genes are frequently methylated in oral malignancies. It is commonly known that deletion and mutation at p53 as well as loss of heterozygosity at 9p21 is a frequent observation in OSCC. The development of cancer has been linked to promoter hypermethylation of a growing number of genes and is observed in various types of tumor eg., Tumor suppressor gene p16INK4a and DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT). Rates of methylation of this promoter in OSCC vary between 31% (30) and 67% (31). The levels of methylated gene differ in value in each tissue and are high in surrounding normal tissue (50%) and leukoplakia (44%). This has led to p16 methylation being previously described as an early change in oral carcinogenesis of little prognostic value.

The reduced expression of E-cadherin has been correlated with regional metastasis in OSCC. It is a trans-membrane glycoprotein responsible for cell–cell adhesion.

Several studies have detected frequent methylation of secreted
frizzled-related protein (SFRP) genes in OSCC patients. The frequent methylation and silencing of SFRP genes in OSCC have suggested that their loss of function contributes to activation of Wnt signaling leading to cell proliferation during oral carcinogenesis. A study has reported that statistical data attributed to methylation of SFRP genes vary largely - SFRP1 (7/17, 41%), SFRP2 (16/17, 94%) and SFRP5 (14/17, 82%) in a panel of OSCC cell lines, as well as in specimens of primary tumors collected from 44 OSCC patients (SFRP1, 10/42, 24%; SFRP2, 16/44, 36%; SFRP5, 7/43, 16%). A recent study using demethylation unmasking of potentially epigenetically silenced genes in HNSSC reported that the CYCA1 promoter was methylated in 45% of tumors but in none of the normal tissues.

MGMT is a detoxifying agent of DNA that addsucts and prevents alkylation. The frequency of MGMT promoter Reports on transcriptional silencing of MGMT gene in OSCC have shown that inactivation of this DNA repair mechanism may be a significant event in oral carcinogenesis.

MGMT methylation frequency in various tissue samples has been studied. Among pre-malignant lesions, the methylation frequency is highest for leukoplakia with dysplasia (73%, \( P = 0.0002 \)) followed by leukoplakia without dysplasia (73%, \( P = 0.0015 \)), SMF (46%, \( P = 0.0437 \)), and OLP (25%, \( P = 0.4386 \) NS). It has also been notified that in case of blood samples methylation was significantly the highest in leukoplakia without dysplasia (55%, \( P = 0.0049 \)) followed by leukoplakia with dysplasia (41%, \( P = 0.0166 \)), SMF (31%, \( P = 0.0821 \), NS), and OLP (25%, \( P = 0.1904 \)NS). In the study of all subgroup of premalignant oral lesions the MGMT gene methylation in the OSCC group was significantly higher (\( P = 0.0379 \) for tissue and 0.0468 for blood samples).

APC gene located at 5q21 chromosome is actively involved in signal transduction pathway. The down-regulation of APC in significant amount has been detected by immunohistochemistry in 15 (30.0%) of 50 tissue samples and by the reverse transcriptase-polymerase chain reaction in five (62.5%). Two of eight (25%) OSCC-derived cell lines exhibit hypermethylation of the APC promoter CpG island. Thus, the APC contributes to the development of human OSCC by about 30%, by down-regulation of its expression. The hypermethylation of the gene promoter CpG islands has been confirmed to be a significant mechanism of inactivation of the APC gene in oral carcinogenesis.

The semi-quantitative RT-PCR analysis showed downregulation of TSC1, TSC2, EIF4EBP1, PTEN and regulation of PIK3CA, AKT1, PDK1, RHEB, FRAP1, RPS6KB1, EIF4E and RPS6 in tumors. Similar observations have been made for AKT1 and RPS6KB1 expression in tumors at the protein level. The mechanism of downregulation of TSC genes have identified LOH in 36.96% and 39.13% of the tumors at the TSC1 and TSC2 loci, respectively. The expression of TSC genes is significantly increased upon treatment of an OSCC cell line with the methyl-transferase inhibitor 5-azacytidine, suggesting methylation of their promoters. However, the treatment of non-OSCC HeLa cells with the 5-azacytidine showed a significant increase in the expression of the TSC2 gene only. All these results conclude that promoter hypermethylation acts an important mechanism for down-regulation of these genes.

In array CGH analysis whole genome was analyzed and through it promoter hypermethylation of PRTFDC1 was found. Through prosequencing study of gene E-cadherin (ECAD), cyclin A1 (CYCA1) and cytoglobin (CYGB) hypermethylation of these are found and methylation indices (MtI) were found as ECAD: 0.702, CYGB: 0.849, CYCA1: 0.812.

In a research by using geneontology (spotfire) and IPA 301 hypermethylated differentially gene were found for their biological significance, among these genes 275 genes expression were found in expression assay. Through methylation-transcriptional silencing analysis, 140 genes were found to be downregulated. Gene were correctly identified in discovery screen and found to be downregulated. Genes like CALCA, HOXA9, GATA4, and NID2 were analysed first time and found to be hypermethylated in OSCC. Promoter methylation of NID2 (\( k = 0.60 \)), KIF1A (\( k = 0.64 \)), HOXA9 (\( k = 0.60 \)) and EDNRB (\( k = 0.60 \)) were also found.

CONCLUSION

Promoter hypermethylation of tumor suppressor gene may be used as diagnostic biomarker in OSCC. This provides the alternative for early detection of the OSCC. This will increase the chance of the patient survival due to detection of OSCC in early stages. Epigenetic changes like hypermethylation are reversible changes unlike genetic changes. These changes may be reversed through the application of various DNA methyltransferase and Histone deacetylase inhibitors. These drugs have shown promising results in cancer therapeutics. Despite of that these drugs show several side effects to the patient. Thus to avoid side effects research is being carried out on several natural compounds from plants which may have potential to cause the reversal of the promoter hypermethylation of the tumor suppressor genes in OSCC.

REFERENCES


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