Review Article

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Dentistry Section

Molecular Predictors in the Early Diagnosis of Oral Cancer

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ABSTRACT

Human beings are being subjected to a variety of disease processes, a majority of which can be cured. However, cancer remains an endangering disease that affects various body parts. Though cancers can be prevented by various therapeutic modalities, the patient survival and the prognosis are questionable, as they are detected at very late stages. The aim of improving the prognosis lies in an early detection of the cancer, which can be brought about by an analysis of the changes in the cellular biomolecules.

Key Words: Cancer, Molecular changes, Growth factors, Loss of heterozygosity, Telomerase, Tumor suppressor genes

INTRODUCTION

Oral cancer is the most common form of cancer which affects the general population worldwide [1]. It is more prevalent in developing countries such as India with the ratio of 1:3 between men and women [2]. The term, 'oral cancer', in a broad sense, refers to the malignancies of the oral tissues, which include odontogenic tumors [3] and salivary gland tumors. However, a majority of the oral cancers are squamous cell carcinomas of the lip and the oral mucosa. A vast number of oral squamous cell carcinomas (OSCCs), at the initial stages, clinically manifest as 'potentially malignant disorders', most often as white or red patches (leukoplakias or erythroplakias, respectively). Erythroplakias are generally considered to have a higher tendency to undergo malignant transformation than leukoplakias (90% or more) [4].

Light microscopy of the premalignant lesions reveals epithelial dysplasia, nevertheless, it has been recognized that occasionally, the non-dysplastic lesions may turn into cancer, while not all the dysplastic lesions become malignant. Therefore, it is highly challenging to identify those lesions that have a true malignant potential [5]. The sub-cellular changes occurring in the tissue are designated as molecular predictors, which helps in early detection of oral cancer offers best prognosis with least invasive treatment.

Molecular changes that predicts the probability of oral cancer

Loss of Heterozygosity (LOH) and hypermethylation of tumour suppressor genes (TSGs) such as p53, p16, p27,[5] Rb, MEN1, APC[6] that occurs in oral premalignant lesions (OPL) silences its action, and will facilitate the neoplastic transformation [7]. Since the cell kinetics (proliferative activity) is analyzed on the basis of expression of Ki-67, PCNA,[8] thymidine labeling index and Mitotic frequency-MPM-2, they are also considered as markers for malignant transformation in OPL [9].

The genes that orchestrate *apoptosis* (Bcl-2 and bax) are extended to the basal layers in an oral dysplastic condition, but recent studies have reported that the bcl-2 expression and the apoptotic index are not of prognostic significance, while there exists some correlation between the bax/bcl-2 expression and the histological grading of OSCC [4]. However Sudbo et al., considered aneuploidy (alteration in DNA content) as a marker of a progressive dysplasia [5].

***Matrix Metalloproteinase* (MMP)** – The higher levels of MMP-1 and 9 in dysplasia are likely to aid a malignant transformation. Co-expression of MMP-11and VEGF are associated with dysplastic progression to OSCC [5]. EMMPRIN (CD_{147}/M6) is a well known extracellular *matrix metalloproteinase inducer*, it mediates a cross talk between the tumour and the corresponding stroma and its expression signifies a cause rather than a consequence of a neoplastic transformation of OPLs [10].

Growth factors such as the epidermal growth factor and its receptor (EGF/EGFR), the vascular endothelial growth factor (VEGF) and the transforming growth factor (TGF) show increased expressions, which signify inverse correlations to the prognosis factor [4,5,8]. Hence, they can be used as targets for therapeutic purposes. HIF-1 and iNOS induce VEGF, thereby favouring the tumour angiogenesis in OPLs [11, 12].

The *cytoskeleton* of the oral epithelial cells (cytokeratin) shows an altered expression or an altered differentiation, which signifies that the dysplastic tissue is approaching a malignant development. The circulating cytokeratin levels in serum could be a valuable aid in assessing the state of the patient before any clinically significant change had occurred. SCCAg, CEA, CA Ag19.9, Neuron specific enolase (NSE) and TPS can be utilized, even for the management of oral cancer [5, 8, 13]. Cyfra 21-1 (the soluble fragment of cytokeratin 19, that is present in saliva) correlates well with OSCC [6].

αvβ6 integrin, an intercellular adhesion molecule of the squamous epithelium, mediates the cellular cross talking. Its primary task is to sustain the tissue integrity and to monitor the function of the cells. The intensity of the expression is elevated in OSCC [5, 14], while the CD_{44} (cell surface glycoprotein) expression is diminished in lesions with a poor prognosis and it facilitates the formation of new tumour [5].

The *blood group antigens* are antigens which are expressed in cells other than erythrocytes and they are collectively known

as "histo-*blood group antigens*". Their expressions, namely-the A,B,H, Lewis (Ley) and the T/Tn systems are altered in OPLs and OSSCs [15]. Whereas the enzyme telomerase that usually prevents the cellular senescence is markedly increased in stem cells and tumour cells. Reactivation of this cause uncontrolled proliferation which is the prime element in conversion and development of tumour [4].

Mitochondrial DNA mutations remain a striking diagnostic marker of the disease process, as they are profusely found in every cell. Hence, they may also be implicated in the targeted cancer therapy [12]. Microsatellites connote the tandem repeats of the nucleotides which are located in the non-coding areas of the genome. The allelic loss can be detected in the mouth washes and the brushings of the neoplastic lesions. The accuracy of this analysis is independent of the tumour stage, thus suggesting that this approach may be useful in an early diagnosis as well as in the follow-up [4].

OTHER MARKERS

The plasma microRNA miR-31, G protein-coupled receptor 87, Rab11 GTPase, PDZ domain containing proteins and PEST-containing nuclear proteins [6] and Annexin A1 (ANXA1) [16] are proposed to be oral cancer markers, while Cyclooxygenase (COX)-derived prostaglandin E2 (PGE2) facilitates tobacco-induced OSCC. There is an increase in C16, C24 and the C24:1-ceramide levels increase and decrease in C18-ceramide in OSCC. Overexpression of Phosphatidylinositol synthase is a marker for early cancer, since it is the of target for smokeless tobacco carcinogens [6].

Hormones are also considered to contribute to the development of OSCC. Studies by Dietrich et al., and Shklar et al., emphasize that females after menopause have defective estrogen synthesis and are at risk of developing OPL while those on estrogen therapy seldom showed any oral lesion. However more studies are required to approve this fact [6].

The ACE gene polymorphism indicates a risk for OPLs and cancer; the genetic alteration is made possible by the activation of chronic inflammatory cytokines, (serum or salivary tumor necrosis factor-a, prostaglandin E2, interleukin-1a, 6, 8) [17]. Detection of the *HPV*16 DNA in plasma can be used for surveillance [7].

The common techniques which detect the molecular changes [4]

1. In situ hybridization
2. Fluorescent In situ hybridization (FISH)
3. Cytomorphometry
4. DNA image cytometry
5. Flow cytometry
6. Immunohistochemistry
7. Polymerase chain reaction
8. DNA microarrays
9. Proteomics

However these premalignant lesions with molecular alterations can be visualized clinically by the utility of Toluidine blue, light-based detection systems such as Chemiluminescence (ViziLite Plus; Microlux/DL), tissue fluorescence imaging (VELscope) and tissue fluorescence spectroscopy [16] which alarm the clinicians. The salivary rinses as well as serum provide good mediums for collecting the genomic content, which aid in the detection and surveillance, in case the lesion is not clinically evident.

FUTURE CONSIDERATIONS

Oral cancer is a commonly prevailing disease among the world's populations. It is not always possible to predict the risk of a malignancy, unless there exist advanced tissue changes which can elicit a clinical symptom. By that time, the patient's survival becomes questionable. The evolution of sophisticated diagnostic techniques can help in studying the biomolecules in the cells and in assessing the state of being healthy and unhealthy. However, till date, there is no definite method which can pinpoint early cancer. This requires lot of experimental studies with respect to the improvement of the prognostic value.

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