Molecular Insight Into Pathogenesis of Potentially Malignant Disorders of Oral Cavity

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Abstract
Early detection can decrease both morbidity and mortality associated with this neoplasm. However, screening for potentially malignant disease is typically confounded by difficulty in discriminating between reactive/inflammatory lesions vs those lesions that are premalignant in nature. Selected markers for cell proliferation, adhesion, apoptosis and lymphocytic infiltration were analysed by immunohistochemistry in addition to static cytometry for DNA content. In the present review, we have discussed the molecular pathogenesis of Oral lichen planus, oral sub mucous fibrosis and Leukoplakia.

Key words: Leukoplakia, Oral lichen planus, Oral sub mucous fibrosis, Precancer.

Introduction
The sixth most common malignancy of the world is Squamous cell carcinoma (SCC) of the oral and oropharyngeal region. Despite numerous advances in treatment, long-term survival from this disease remains poor. Early detection can decrease both morbidity and mortality associated with this neoplasm. However, screening for potentially malignant disease is typically confounded by difficulty in discriminating between reactive/inflammatory lesions vs those lesions that are premalignant in nature.1 Furthermore, the histologic diagnosis of dysplasia can be subjective and is thus prone to a considerable range of interpretation. Similarly, no definitive, validated criteria exist for predicting which dysplastic lesions are most likely to progress to cancer over time. Given this state of science, the presence of dysplasia can only be used to indicate that an oral lesion may have an increased risk of malignant transformation. Molecular biomarkers capable of identifying the subset of lesions likely to progress to cancer are required to eliminate this clinical diagnostic dilemma.2

Oral Sub Mucous Fibrosis
Oral submucous fibrosis (OSMF) has also been previously described as idiopathic scleroderma of mouth, idiopathic palatal fibrosis, sclerosing stomatitis and juxta-epithelial fibrosis.3 The hallmark of the disease is submucosal fibrosis that affects most parts of the oral cavity, progressive trismus due to rigid lips, cheeks, pharynx and upper third of the esophagus leading to dysphagia. The disease is mainly seen in Asian countries and the prevalence is more in India. OSMF was first reported by Schwartz in 1952 while examining five Indian women from Kenya, which he called as “atrophiacidiopathica (tropica) mucosae oris”.4, 5 Later in 1953, Joshi from Mumbai re-designated the condition as OSMF, implying predominantly its histological nature. Its precancerous potential was first reported by Paymaster in 1956. Rao in 1962 suggested that OSMF is a localized condition of collagen disease.6

Molecular pathogenesis
The three main events that are modulated by TGF-b, which favors the collagen production are: (1) activation of procollagen genes; (2) elevation of procollagen proteases levels: (a) procollagen C-proteinase (PCP)/ bone morphogenetic protein1 (BMP1) and (b) procollagen N-proteinase (PNP); (3) up-regulation of lysyl oxidase (LOX) activity.6

Collagen is the most abundant protein in the human body and it plays a critical role as a structural element of connective tissue. About 27 types of collagen have been recognized, which can be grouped into seven broad classes. Major class is fibrillar collagen, among them types I, III, and VI form a major part of connective tissue. Collagen type VII forms the anchoring fibrils of oral mucosa.7 8 The distinguishing feature is a unique type of triple helix, stabilized by unusual cross-links. The processing of fibrillar collagen occurs in a...
The LOX is an essential enzyme for final processing of collagen fibers into a stabilized covalently cross-linked mature fibrillar form that is resistant to proteolysis. The LOX is dependent on copper for its functional activity. Removal of copper leads to a catalytically inactive apoenzyme. The LOX is synthesized as prolyl oxidase and conversion of this precursor into an active LOX is mediated by BMP1 and takes place in the extra cellular space. During the biosynthesis of LOX, copper is incorporated into LOX. Apart from copper, LOX also contains another co-factor, a covalently bound carbonyl prosthetic group – lysine tyrosylquinone (LTQ). The LTQ is essential for the reaction mechanism of LOX, i.e. in the formation of cross-links in the collagen fibers. Copper has been suggested to play a structural role in stabilizing the LTQ. During the process of cross-linking, copper plays an important role in reoxidizing the reduced enzyme facilitating the completion of the catalytic cycle. Areca nuts have been shown to have a high copper content, and chewing areca nuts for 5–30 min significantly increases soluble copper levels in oral fluids. This increased level of soluble copper could act as an important factor in OSMF by stimulating fibrogenesis through up-regulation of LOX activity. Apart from this, the flavonoids that are present in areca nut have been implicated in the process of enhancing the cross linking of collagen fibers. In vitro studies have demonstrated the presence of catechin to raise LOX activity. They might be oxidatively converted to quinones and hence, might resemble LTQ, which is an important co-factor for LOX activity. This could be a possible explanation for enhancing LOX activity. Apart from this process, the in silico, molecular modeling experiments have revealed that the direct interaction of flavonoids with collagen facilitates the cross linking of collagen fibers.

The expression of LOX is regulated by various factors, among which TGF-b is considered to be an important factor. TGF-b has been found to strongly promote the expression of LOX both at the mRNA and protein levels in various cell lines. The exact mechanism underlying this is not yet fully understood. This could be indirectly via the elevation of BMP1 by TGF-b, as it mediates the biosynthetic processing of LOX, i.e. conversion of prolyl oxidase to active LOX. The LOX activity is important for formation of insoluble collagen due to cross-linking. The process of cross-linking gives tensile strength and mechanical properties to the fibers as well as makes the collagen fibers resistant to proteolysis. Increased levels and activity of LOX due to increased BMP and copper levels, and further enhancement of its activity by LTQ like flavonoids present in BQ, causes increased crosslinking of the collagen fibers, tilting the balance towards a fibrotic condition as present in OSMF.

**Oral Lichen Planus**

Lichen planus is a chronic inflammatory disease that affects the skin and the mucous membrane. Oral lichen planus (OLP), the mucosal counterpart of cutaneous lichen planus, presents frequently in the fourth decade of life and affects women more than men in a ratio of 1.4:1. The disease affects 1–2% of the population. It is seen clinically as reticular, papular, plaque-like, erosive, atrophic or bullous types. Intraorally, the buccal mucosa, tongue, and the gingiva are commonly involved although other sites may be rarely affected. Oral mucosal lesions present alone or with concomitant skin lesions. The skin lesions present as violaceous flat-topped papules in ankles, wrist, and genitalia, but characterizedly the facial skin is spared.

The etiology and pathogenesis of OLP has been the focus of much research, and several antigen-specific and nonspecific inflammatory mechanisms have been put forward to explain the pathogenesis. Although mostly palliative, a spectrum of treatment modalities is in practice, from topical application of steroids to laser therapy.

**Molecular pathogenesis**

Abnormal nuclear DNA content (aneuploidy) is an indicator of chromosomal aberrations and is associated with malignant and premalignant lesions. In oral precancer studies, DNA index measurement is thought to be more suitable for risk assessment of an identified precancerous field and of less value in early diagnoses despite the higher analytical sensitivity. Aneuploid dysplastic lesions are shown to develop SCC in a shorter period than diploid; thus, measurement of DNA index might be valuable to determine the time to cancer progression.

Based on the studies, the 2.5c exceeding rate (ER) and the proliferation index of DNA content are shown to be useful parameters in predicting malignant transformation. With more strict criteria defined by Auer et al., aneuploidy is classified as 2.5cER more than 35% and 5cER valued over 0%. These results are compatible with the figures on potential risk of cancer development in OLP (0.46.25%). In nuclear DNA content studies, many cellular parameters can be detected with static cytometry analysis. Former studies showed that in prostate and cervical carcinomas, the G2/M phase is a strong prognostic marker in cancer development.

A few additional reports exist on epithelial DNA content measurements including OLP biopsies and cytology. The results are conflicting and DNA content varies from diploid to aneuploid DNA.
content in OLP. The most important difference among these studies seems to be the method used in DNA content measurement. In certain studies, cell separation technique was used for the image cytometry measurement. In another study, exfoliative cytology samples from OLP lesions were used. In static cytometry, both the morphology and exact location of the measured cells can be assessed simultaneously. Another distinct difference is that most of the authors used the reticular form of OLP which is the most unlikely form for malignant transformation. However, there is one previous study where few erosive OLP lesions were classified as aneuploid; hence aneuploid changes and DNA cytometry can be suitable screening methods for OLP to detect the high-risk lesions.

Cell cycle arrest helps in maintaining tissue integrity and facilitating DNA repair mechanisms; however, at the same time, entry into senescence could favor malignant transformation. Inactivation of p53 is a frequent phenomenon in OSCC. This is caused by mutations, presence of HPV virus and other molecular alteration occurring in the p53 pathway.

As p53 expression has been identified as a response to DNA damage, the identification of p53 in OLP tissue is interpreted as an indication of precancerous potential by some researchers. In support to this concept, Chaiyarit et al. showed an inducible nitric oxide synthase-dependent DNA damage and p53-elevated expression in OLP patients. Another concept is that the high expression of p53 in OLP is a result of the higher cellular proliferation. To prove that p53 expression in OLP is not just a result of the inflammatory process, Safadi et al. compared the immunohistochemical expression of p53 and its downstream effector p21WAF1 between OLP and other inflammatory oral conditions and found significantly higher expression in OLP.

**Leukoplakia**

The word leukoplakia means white patch (leuko-white, plakia-patch). It is considered as the premalignant lesions, but now included in a broader term for common usage of tobacco in the form of smoking and chewing. High-risk of malignant transformation is encountered if the risk factors are not eliminated. It has been reported that many oral squamous cell carcinoma develops from the potentially malignant disorders. Correct diagnosis and the right treatment at right time of potentially malignant disorders may prevent malignant transformation of these lesions.

**Molecular pathogenesis**

Loss of heterozygosity in a cell is the loss of normal function of the allele of a gene whose homologous allele was previously inactivated. This prior deactivation occurs in parental germ cells and is transmitted to their offspring to generate cells that are heterozygotic for the gene in question. The development of this phenomenon in regions of the chromosome with tumor suppressing genes could be related to the process of malignant transformation. The loss of heterozygosity in oral leukoplakia and its possible predictive value have been reviewed recently by Zhang and Rosin, who establish that lesions with such loss limited to chromosomes 3p and/or 9p would form part of the group of leukoplasias of intermediate risk, with a 3.8-fold increase in risk of malignant transformation, whereas lesions with loss from 3p and/or 9p and loss from one or more of the 4q, 8p, 11q, 13q, and 17p chromosomes would be considered as high-risk leukoplasias, with a 33-fold greater risk of progression to cancer. Finally, low-risk leukoplasias are considered those that do not present any of the above losses of heterozygosity. Study of these molecular alterations in resection margins complements histological information and can demonstrate the presence of molecular abnormalities at borders that are histologically free of disease—an observation which could explain recurrences and the development of oral squamous cell carcinoma. In fact, molecular confirmation of complete resection of precancerous lesions was shown to be strongly correlated with a decreased risk of oral carcinoma in patients with high- or intermediate-risk leukoplasias, in contrast to low-risk forms where no such correlation was found.

Microsatellites are repeats of non-coding DNA sequences that occur normally within the human genome. Defects in the DNA repair process can lead to microsatellites that are abnormally short or long; this process has been termed microsatellite instability (MI). MI is indirect evidence of an abnormal mismatch repair (MMR) protein’s function (hMLH1, PMS2, MSH2, MSH6). A proposed mechanism relevant in Squamous cell carcinoma of head and neck region (SCCHN) tumorigenesis is through promoter hypermethylation. When MMR promoters are hypermethylated, it provides indirect evidence of a higher chance that promoters of tumor suppressor genes are hypermethylated too, and therefore nonfunctional. Alternatively, when a microsatellite repeat replication error goes uncorrected, a germ line hereditary mutation could result leading to inactivation of tumor suppressor genes and uncontrolled cell and tumor growth. This concept of a mutator phenotype provides an alternative to a multistage accumulation of genetic alterations to explain head and neck tumorigenesis. Specifically, the loss of function of a gene critical for the repair of DNA damage greatly increases the mutation rate at other loci leading to genome-wide instability.

In a study of 93 premalignant and 18 invasive SCCHN cases, an increasing trend of MI was found from hyperplasias (6% of specimens) to dysplasias/CIS (27%) and to invasive cancers (33%) [13]. A similar trend was found in another study where 15% of dysplasias and 30% of invasive cases manifested MI at multiple loci. Partridge et al. found as high as 55% of 31 leukoplasias and erythroplakias to show MI. The incidences of MI found in these and other studies in head and neck malignancies are significantly higher than those reported in breast, skin and non-small-cell lung cancers. The prevalence of MI appears to vary between tumor types.

Telomerase is an enzyme with polymerase activity formed from a protein-RNA complex. It is produced in embryonic germline cells and its function is to lengthen the telomeres by copying the TTAGGG sequence. Telomerase plays an important role in the formation, maintenance, and renovation of telomeres, preventing cell apoptosis. It is suppressed by mature somatic cells after...
birth, allowing telomere shortening after each cell division. Overexpression of telomerase has been reported to be associated with a range of neoplastic diseases. The human telomerase reverse transcriptase (hTERT) gene encodes the catalytic subunit of telomerase and shows a positive correlation with telomerase activity in different molecular studies. Overexpression in leukoplakia, associated with increased telomerase activity, is an early phenomenon in the process of oral carcinogenesis and one that can be detected in precancerous stages. This phenomenon shows a marked positive correlation with the degree of atypia, showing severe dysplastic changes.

Califano et al. tested ten most common allelic events in a large number of primary pre-invasive lesions and invasive HNSCC to develop a molecular progression model. It involves inactivation of many putative suppressor gene loci. Chromosomes 9p and 3p appear to be lost early, closely followed by loss of 17p. Mutations in p53 gene are seen in the progression of pre-invasive to invasive lesions. Many other genetic events occur later during progression. Other genetic events, such as amplification of cyclin D1 and inactivation of p16 have been tested predominantly in invasive lesions, but their precise order in the model was not determined.

The pattern of specific gene mutation in OC patient may give a clue to the aetiology of that particular tumor. Brennan et al. analyzed the pattern of p53 mutation in HNSCC. They found that the incidence of p53 mutation was much higher in patients who were exposed to both tobacco and alcohol versus non-users. It has been suggested that alcohol appears to augment the effect of smoking due to an increase in the absorbance of carcinogens contained within the cigarette smoke. Several epidemiologic evidences suggest that abstinence from cigarette smoking may decrease the overall incidence of HNSCC.

HPV positive oral and oro-pharyngeal cancer comprise a distinct clinico-pathological entity. They are less likely to occur among heavy smokers and drinkers, have lesser likelihood of p53 mutation and have better cancer-specific survival. It has been suggested that HPV positive tumours may have better prognosis by inactivating retinoblastoma (Rb).

**Conclusion**

Understanding the pathogenesis of the premalignant pathologies is very important for planning the treatment protocol. Promising technologies are being rapidly developed to assist in localization of abnormal oral mucosa, in noninvasive and objective diagnosis and characterization of identified mucosal lesions, and in therapy of patients with potentially malignant disorders.

**References**


