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Integrative analysis of DNA microarrays in orofacial premalignant and malignant lesions (a review)

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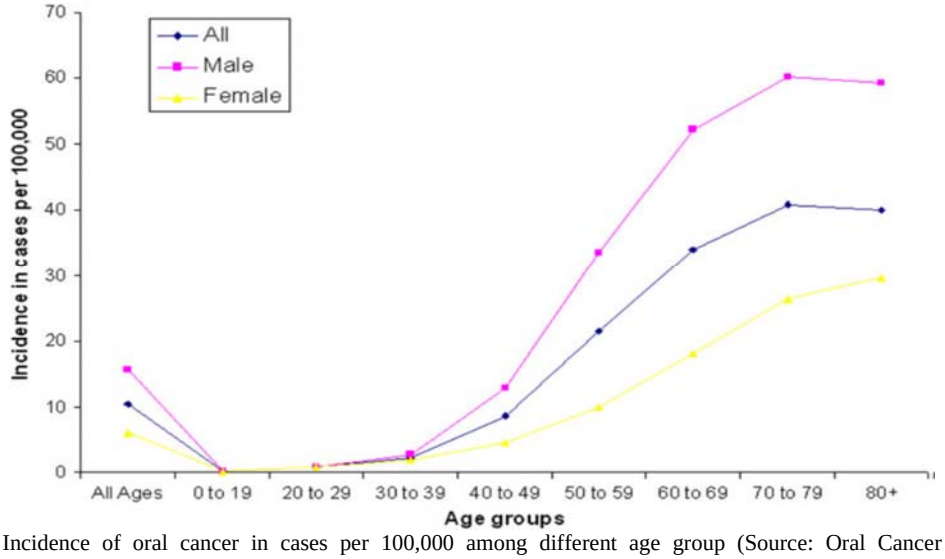
Abstract
Introduction; Head and neck cancer is the sixth most common type of cancer worldwide; it constitutes a major health problem particularly in developing countries. Cancer biomarker plays very important role to detect possibility of transformation of premalignant to malignant, facilitate drug development, improve staging of patients, and predict patient prognosis. Because cancer is the result of many interacting genes, analysis based on a set of genes with related biological functions or pathways may be more informative than single gene-based analysis for cancer biomarker discovery. The relevant pathways thus identified may help characterize different aspects of molecular phenotypes related to the tumor.
Conclusion: Present study helps to prevent transformation of premalignant lesion to malignant lesion and further morbidity by using cancer biomarkers.

Keywords: DNA microarrays, orofacial premalignant, malignant lesions

Introduction

Oral cancer (OC) is the commonest cancer in India, accounting for 50–70% of total cancer mortality and accounts for highest incidence among Asian countries [1]. OC is the sixth most common cancer worldwide. It affects anterior tongue, cheek, floor of mouth, gingiva or any other part of the oral cavity [1, 2].

Worldwide, there is a great variation in the incidence of cancer of the oral cavity. It accounts for less than 5% of all cancers in United States, Western Europe and Australia. India, few pockets in France, Brazil, central and eastern Europe have few of the highest rates of cancer of the oral cavity in the world [1, 3].



Incidence of oral cancer in cases per 100,000 among different age group (Source: Oral Cancer Incidence by Age, Race, and Gender. Data accessed from National Institute of Dental and Craniofacial Research.

The incidence of OC is directly correlated with age of subjects. Rates rise dramatically after the age of 40–49 years, and reach a plateau around the age of 70–79 years (Fig. 1). Due to increase in ageing population in majority countries world-wide, even the current age specific rates will be more than sufficient to tilt the balance towards an increase in adult population with higher risk of OC. OC is more frequent in men than women, and depending on its location within the oral cavity, males are two to six times more likely to be affected than females, largely owing to their higher intake of alcohol and tobacco [1].

2. Etiology and Major Risk Factors

Tobacco, alcohol, high-risk human papillomavirus (HPV) types 16, 18 and genes like P^{53} protein, Ki-67 antigen, Allelic imbalance (AL) at chromosome 3p, 9p, 11q and 17q, P^{16} protein, over expression of EGFR, Cyclin 01, Matrix metalloproteinase are considered to be associated risk factors for the development of head and neck cancer, acting as potent carcinogens leading to malignant transformation [1, 4, 5].

3. Importance of Early Treatment of Premalignant Lesions Predisposing To Oral Cancer

Not only oral cancer, premalignant lesions like leukoplakia, submucous fibrosis, atrophic candidiasis, sever iron deficiency, syphilitic lesions, long standing traumatic ulcers should treat early and advice for biomolecular test, by which we can prevent transformation of premalignant to malignant lesion and associated morbidity.

Attention should be paid specifically to precancerous lesions. Leukoplakias are among the most common potentially malignant oral lesions. Some are idiopathic, while others are related to habits such as tobacco, alcohol or betal quid use [4, 5]. About 80% of leukoplakias are benign, with no evidence of dysplasia and no tendency to malignancy, but biopsy is clearly indicated to define the remaining 10–20% that are either dysplastic or already changed to invasive carcinoma [1, 4]. Overall, the rate of malignant transformation of leukoplakias is about 3–6% over 10 years, but rates that are much higher have been reported [4]. Medical management of leukoplakias includes reducing or quitting those habits relating to risk factors, increasing the intake of fruits and vegetables in the diet, and possibly the use of active agents [7]. Retinoids, carotenoids and topical cytotoxic agents inducing apoptosis show promise, and newer therapies are on the horizon [1, 4]. Also, matrix metalloproteinase-13 (MMP-13) could be a potential tumor marker for OSCC. Epigallocatechin-3-gallate, a component of green tea, has also been demonstrated to inhibit oral cancer through the modulation of MMP-1[6].

4. Molecular Pathogenesis of Oral Cancer

Oral carcinogenesis like any other cancer is a progressive disease and normal epithelium passes through stages starting from dysplasia to finally transforming into invasive phenotypes [1]. Although all types of carcinomas are seen in oral cavity, the most common form of OC is squamous cell carcinoma [1, 7].

Oral cancer is also considered to be a multi-hit progressive disease and normal epithelium passes through stages starting from dysplasia to finally transforming into invasive phenotypes [4]. Involving a number of aberrant genetic events culminating in malignant transformation at the molecular and biological levels [1, 4]. It is known that following the action of various carcinogens (chemical, physical, biological) on normal cells in humans, a long period (latency) of several months to years (~10 months to 30 years) occurs between the development of precancer cells and their transformation into cancer cells [1, 5, 8].

Use of genetic and proteomic approach in recent years have revealed the molecular pathological picture of OC. There is active search to identify genetic alterations in oncogenes or tumour suppressor genes, role of genomic instability and epigenetic modifications and to generate a gene expression profile in oral oncogenesis [6].

However, the molecular and biological events that take place within the precancer cells during this quiescent stage are not yet fully understood. Recent studies revealed that preneoplastic cell development and transformation into cancer cells is determined initially by genetic changes (oncogenes, antioncogenes), with sequential multiple somatic mutations, and later by epigenetic or environmental cell factors such as hormones, growth factors, cytokines, vitamins, and prostaglandins [7, 9]. These factors can markedly change the evolution of preneoplastic cells by enhancing, retarding, or inhibiting their transformation into cancer cells, or even reversing them to a normal phenotype [7]. These effects act on DNA, RNA, and protein synthesis, as well as on cell replication, cell cycles, cell surfaces, and intercellular communication [7].

Therefore, these abnormal DNA, oncogenes or tumor suppressor genes, and ultrastructural intracellular or cell surface antigenic determinants as potential biomarkers are essential for early detection of preneoplastic cells and cancer cells [5]. A significant recent advance is the gradual understanding of the molecular mechanism of oral cancer formation. Although a universal tumor marker is still lacking for oral cancer, a combination of several markers may be useful and more accurate [5, 7].

5. Genetic Susceptibility

It is now established that up to 10% of all cancers have a strong hereditary component. Role of genetic component in the development of OC is being suggested by several studies showing familial clustering [4]. A clustering of OC has been seen in certain ethnic groups, like Ashkenazi group in Israel; with incidence being double as compared to other Jewish population in that country. However, the basis of this genetic susceptibility is not well understood, as yet [1, 3, 9].

Evaluation of specific genetic polymorphism in key genes involved in oral carcinogenesis has been the major area of study. Glutathione S-transferase M1 (GSTM1) null genotype appears to be the most consistent polymorphic susceptibility marker for head and neck cancer including OC. Meta-analyses by Tripathy and Roy showed that the GSTM1 null genotype conferred a 20–50% significantly increased HNSCC risk [1, 4]. The variant val allele of the CYP1A1 (Cytochrome P450, family 1, member A1) polymorphism is another fairly consistent susceptibility marker with a 35% increased risk in a meta- analysis of 12 studies. The studies on many other gene polymorphisms have been inconclusive. Brennan *et al*. found that ALDH1B and ALDH2 (Aldehyde dehydrogenase 2) genes were associated with HNSCC and showed significant correlation with alcohol consumption [1].

Proto-oncogenes, Oncogenes and Genetic Alterations: Genetic alterations define molecular basis of carcinogenesis which includes point mutations, amplifications,

rearrangements, and deletions [4]. Several oncogenes have also been implicated in oral carcinogenesis. Aberrant expression of epidermal growth factor receptor (EGFR), K-ras, c-myc, int-2, Parathyroid adenomatosis 1 (PRAD-1) and B-cell lymphoma (bcl) like oncogenes have been reported in OC development. Over expression and amplification of cellular oncogene EGFR have been reported in a [7], 12-Dimethylbenz (a) anthracene (DMBA) induced hamster cheek pouch malignant OC model [1, 7, 10].

Transforming growth factor-alpha (TGF-a) is known to promote neovascularization and mitogenesis. It has been shown to be aberrantly expressed in human OC and in hamster oral tumor [10].

p16 and p53

It has been established that the proto-oncogene, cyclin D1, is amplified in head and neck squamous cell carcinoma (HNSCC) as well as in other malignancies, and one of the most common deleted gene loci in head and neck cancers occurs at chromosome 9p21-22. This critical deletion includes the p16 region, a tumor suppressor gene, which suppresses cyclin D [1, 3].

Therefore, any deletion or mutation occurring on p16 leads to cyclin D1 amplification, which is most frequently encountered in various HNSCCs. Homozygous deletions or methylation in the 5' CpG region of p16, which causes the complete inactivation of p16 through the blockage of transcription, have been identified in approximately one-fourth of primary head and neck cancers [5].

Mutations in the tumor suppressor gene, p53, located on chromosome 17q13, are observed in approximately 50% of head and neck cancers [7, 8]. These mutations result in elevated dysfunctional p53 levels, a phenomenon secondary to loss of negative regulation via Mdm2 (murine double minute oncogene 2), a finding noted in tobacco users. In a study conducted by Field *et al*, heavy smokers showed elevated dysfunctional p53 ($p<0.005$) when evaluated for squamous cell carcinoma of head and neck. The loss of function of p53 leads to uncontrolled growth, making cells incapable of appropriately responding to DNA damage or stress [7, 8].

Moreover, tobacco usage has been demonstrated to cause Ras (Rat sarcoma*)* mutations, as identified by studies in India and Taiwan, who have an enormous population of tobacco chewers. Of the Ras mutations, H-Ras has been implicated in HNSCC and is associated with tobacco chewing. Notably, mutations of H-*Ras* have been proven to be favorable in terms of prognosis, while low levels of H-Ras expression result in poor prognosis [6].

In HPV-16-related malignancy, the E6 protein is known to inactivate the p53 gene via ubiquitination, disturbing the cell cycle and DNA repair mechanisms [6].

Genomic Instability

Genomic instability such as loss of heterozygosity (LOH) and microsatellite instability (MSI) are frequently observed in cancer and such instability has been investigated and several reports are available in OC. Chromosome 9p21 containing p16 tumor suppressor gene is frequently lost in HNSCC and oral preneoplastic lesions [1, 7]. Chromosome 3p14 contains the tumor suppressor gene fragile histidine triad (FHIT) as well as a common fragile site, FRA3B which is also found to be frequently deleted in early tumorigenesis and its deletion is associated with exposure to cigarette smoke. Loss of chromosome 17p13 harboring p53 tumour suppressor gene is also common in multistep head and neck tumorigenesis [1, 7]. Loss of function of the tumour suppressor p53 can result in uncontrolled cell division and progressive genomic instability. The increased frequency of LOH in invasive tumours at the 9p21 locus is also reported and may suggest that the region, probably the p16 gene is important in early malignant progression [7].

Epigenetic Alterations

The major epigenetic modification in tumours is methylation. Changes in the methylation patterns can play an important role in tumorigenesis [4, 5]. Epigenetic modifications are frequently connected with the loss of genetic expression and important for the multiple indispensable genetic events during carcinogenesis. Malignant progression takes place because these alterations can inactivate DNA repairing genes [5, 7].

Methylation patterns of p16, methylguanine-DNA methyltransferase (MGMT) and Death-associated protein kinase (DAP-K) genes in smears of patients suffering from head and neck cancer showed abnormal hypermethylation patterns by a methylation specific polymerase chain reaction (PCR) [5]. 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 [5].

Other genetic events, such as amplification of cyclin D1and inactivation of p16 have been tested predominantly in invasive lesions, but their precise order in the model was not determined [7].

Molecular Epidemiology

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 [5, 7].

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) [1, 7].

New Markers and Tools for premalignant and malignant lesions of oral cavity

The last 10 years has seen a shift in diagnostic methods from the histopathologic to the molecular level. With advances in modern molecular biology techniques, many new markers for oral cancer have been found and studied. Significant momentum has been seen in the exploration P^{53} protein, Ki-67antigen, Allelic imbalance(AL) at chromosome 3p,9p,11q and 17q using microsatellite cancer, HPV16 and HPV18, Increase expression of cytokeratins (CKs) CK 8,18 and CK19,P^{16} protein, Over expression of EGFR, Cyclin 01,Matrix metalloproteinase [7, 9].

6. Conclusion

Increasing knowledge of molecular genetic alterations in premalignant and malignant lesions of oral cavity has led to a better understanding of molecular pathways in the development of OC. This new knowledge is expected to generate new lead for prevention, early diagnosis and devising new therapy for OC.

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