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Inflammation in Oral Cancer and Role of COX-2: A Review.

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ABSTRACT

Oral cancer is 1 of the 10 most frequent cancers in the world. It accounts for approximately 2% of all cancers and 1% of all cancer deaths. Squamous cell carcinoma is the most common malignant tumor of the oral cavity, accounting for over 90% of the malignant neoplasms in this region, and is thought to arise from a progressive dysplasia of the oral mucosa. Oral squamous cell carcinoma (OSCC) has a multifactorial etiology. Inflammation is a recently defined contributor of oral carcinogenesis. One of the important association of this is Cyclooxygenase (COX)-2. Cyclooxygenase -2 is induced by stimuli such as mitogens, cytokines, growth factors and tumor promoters, and has been elucidated to be up-regulated not only at the sites of inflammation but also in various cancer tissues such as colon, stomach, breast, lung and head and neck including oral cavity. In this paper, we briefly elaborate on the role of inflammation and COX - 2 in the oral cancer.

Keywords: Oral cancer, Inflammation, COX-2, DNA damage, Reactive oxygen species, Premalignant disorders

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INTRODUCTION

Oral squamous cell carcinoma has a multifactorial etiology. Etio-pathogenesis of oral cancer comprises of factors like genetic, environmental and gene-environment interactions, viral and behavioural (smoking, alcohol).[1]

Oral carcinogenesis, a complex and multi-step process, is thought to result from the progressive accumulation of genetic lesions at quantitative or qualitative levels. Deregulation of many genes like oncogenes and tumor suppressor genes has been associated with oral carcinogenesis.[2] The alterations have been traditionally revealed by the use of cytogenetics, immunohistochemistry, or molecular approaches based on one or a few genes. The exact affected nature of each molecule in oral carcinogenesis remains largely elusive.

Inflammation is a crucial, complex host defense against biologic, chemical, physical, and endogenous irritants. The contribution of inflammation to physiological and pathological processes such as wound healing and infection needs to be understood for a better understanding of the role of inflammation in cancer formation.

The majority of patients with oral cancer are presented with advanced-stage disease, mostly because of their nonspecific clinical symptoms, especially in younger patients without traditional risk factors,[3] thus highlighting the necessity of identifying clinically relevant biomarkers of susceptibility for early detection and targeted chemoprevention. The rate at which premalignant disorders (PMDs) transform into oral malignancies ranges between 11% and 36%, depending on the geographic location of the individual and the length of follow-up.[4]

In this paper we are emphasizing on role of inflammation and cox2 in initiation, promotion and progression of oral cancer.

INFLAMMATION & ORAL CANCER

Epidemiological studies have shown that chronic inflammation is associated with various types of cancer.[5] About 15–20% of all deaths from cancer worldwide are linked to infections and inflammatory responses as estimated from the previous researches.[6] It's been more than 100 years that pathologists knew that almost all tumors are accompanied by inflammatory cells. At present, there is almost unanimous agreement about the causes. The functional association dates back to Virchow, who in 1863 hypothesized that cancer arises in sites of inflammation.[5] Today it is accepted that chronic inflammation resulting from low grade, persistent chemical, bacterial, viral agents predisposes the formation of the preneoplastic foci and promotes tumor development.[6] Infectious agents such as *Helicobacter pylori*, with its strong association to gastric cancer, or the relationship of non-infectious chronic inflammation like chronic pancreatitis to pancreatic cancer[5,7] are examples of infection and inflammation leading to tumor growth. Various researches on chronic inflammation which is caused by infections and chronic irritations are being carried out to locate the exact mechanism that triggers the cancer.

Inflammation is part of the host response to either internal or external stimuli[10] which is briefly elaborated following flowchart in the fig-1. Acute inflammation evades early but chronic inflammation persists for a long time and may cause a plethora of diseases including osteoarthritis, asthma, atherosclerosis, inflammatory bowel disease, psoriasis, crohn's disease, ankylosing spondylitis and cancer. Chronic inflammation may cause cancers of different organs including stomach, colon, breast, skin, prostate, and pancreas. It may also induce neoplasia through the increased production of reactive oxygen and nitrogen species, which results in elevated DNA damage.[8]specific transcription factors are the link between cancer and inflammation which once activated have the capacity to increase the expression of genes that are common to both the regulation and the production of mediators of inflammation, and also to the regulation of the survival and proliferation of cancer cells. Chronic inflammation may induce the expression of multiple tumor-promoting genes (such as the tumor necrosis factor gene TNF, the matrix metalloproteinases or MMP genes, and the vascular endothelial growth factor or VEGF genes) that contribute to enhanced cellular migration and angiogenesis through the regulation of the proinflammatory gene nuclear factor - κ B. [8-10] It has been reported consistently that COX-2 is overexpressed in multiple malignancies and precursor lesions, including oral cancers and premalignant disorders (PMDs).[11,12,13]

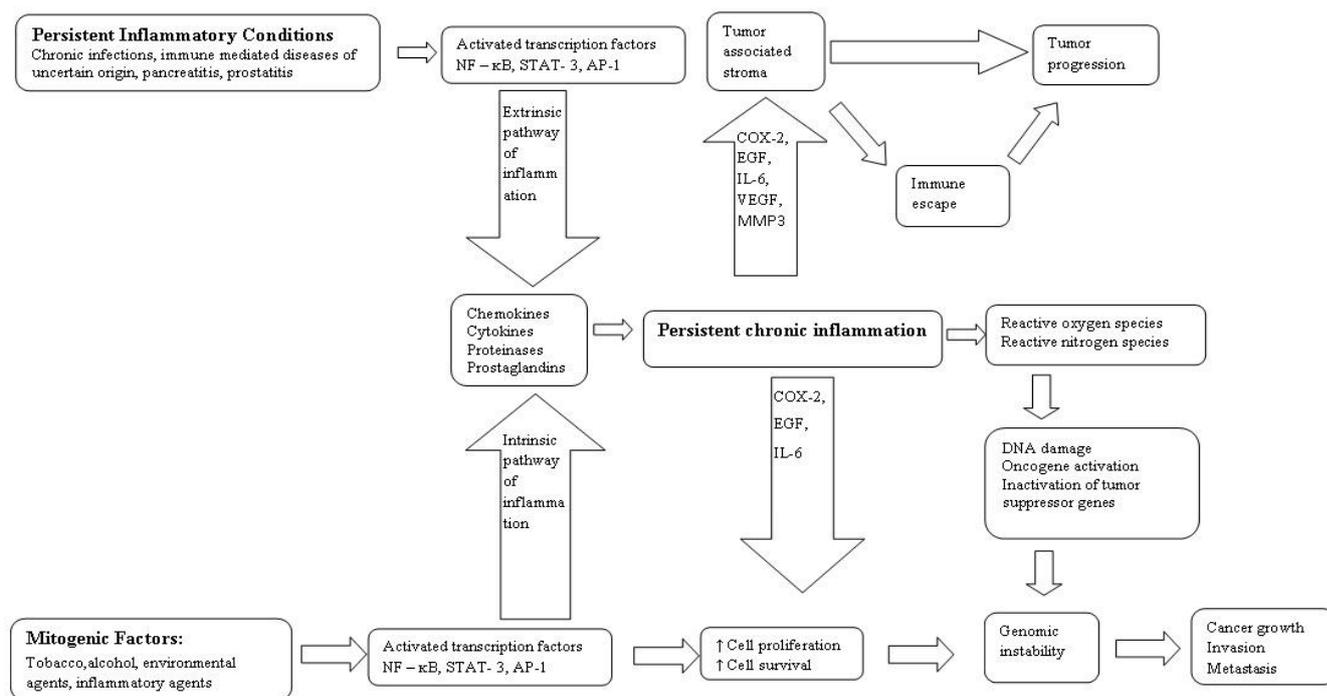


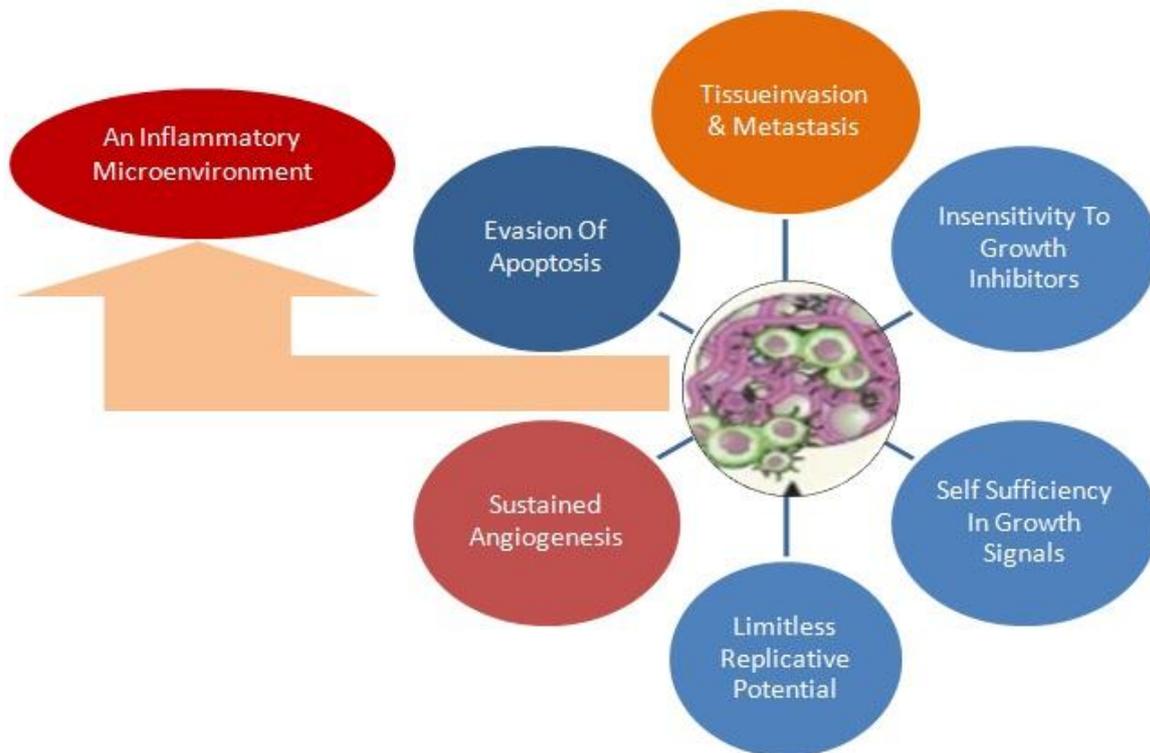
Fig-1: Flow chart depicting the link between inflammation and cancer. In the intrinsic pathway, activated transcription factors that regulate both oncogenic circuits and inflammation related programs, drive the process of tumour development. In the extrinsic pathway, pre-existing inflammatory conditions may favour the onset of cancer and promote tumour progression. Also demonstrates inflammation induced by mitogenic factors and progression of cancer (Intrinsic pathway).

Moreover, selective inhibitors of COX-2 enzymatic activity have demonstrated promising therapeutic potential in the treatment of oral cancers through the inhibition of multiple COX-2-induced oncogenic pathways.[12,14-16]

COX-2 is expressed mainly in response to inflammatory and mitogenic stimuli as shown clearly in the above diagram. As it has already been mentioned earlier that COX-2 is an important enzyme in PGE-2 biosynthesis; it is expressed at high levels in different types of epithelial malignancies and literature review has shown that its expression is associated with poor prognosis. COX-2-derived PGE-2 activates intracellular oncogenic pathways by direct interaction with the transmembrane G protein-coupled receptors EP(1-4), and by transactivation of epithelial growth factor receptor (EGFR), resulting in prolonged cell survival, increased cell proliferation and migration, and in the associated neoangiogenesis. Thus, a positive feedback loop is created whereby COX-2 ultimately upregulates its own expression resulting in increased production of PGE-2, perpetuating a malignant cycle.[71] Inflammation is considered as the 7th hallmark of cancer.[17] Here is a diagram depicting the events in the inflammation leading to tumor progression.

Dysregulated inflammatory response has been found to be associated with most of the chronic diseases, including cancer. The identification of transcript factors such as NF-κB, AP-1 and STAT3 and their gene products such as tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), chemokines, cyclooxygenase-2 (COX-2), 5 lipooxygenase, matrix metalloproteases (MMP) and vascular endothelial growth factor (VEGF), adhesion molecules and others has provided the molecular basis for the role of inflammation in cancer. These inflammatory pathways are activated by tobacco, stress, dietary agents, obesity, alcohol, infectious agents, irradiation, and environmental stimuli, which, combined, account for as much as 95% of all cancers.[18]

Fig 2: Events in the tumor progression. Inflammation is highlighted as one of the proposed responsible factor.



Components of areca nut (AN) are thought to stimulate PGE2 and prostacyclin production by gingival keratinocytes (GK) with concomitant induction of Cox-2 mRNA and protein production.[19] Since it is observed that PGs are important for the initiation, promotion and progression of chemical carcinogenesis.[20] PGs can suppress the humoral and cellular immune action responsible for the killing of malignant cancer cells.[20,21] It appears that the role of Cox-2, TNF- α and IL-6 in the oral carcinogenic processes is complex, intricate and needs to be further addressed. Clinical studies also support the crucial roles of PGE2, IL-6 and TNF- α in head and neck cancer.[22]

Thus we can now predict that in established cancers, there is development of an exaggerated inflammatory stroma which is induced by the cancer cells, which in turn promotes cancer growth, invasion and metastasis. Inflammatory cells of myeloid origin in the tumour-associated stroma, mediate suppression of immune responses against cancer cells, which further favours tumour growth. There are a number of oral inflammatory conditions that have been proposed as being implicated in the pathogenesis of oral squamous cell carcinoma: oral submucous fibrosis, oral lichen planus, discoid lupus erythematosus, oral ulcers related to repetitive tissue injury and chronic periodontal disease. Here we are not dealing with each one of these individually as it is beyond the scope of this paper. Some research studies have found out a positive link between oral inflammation in above mentioned oral inflammatory conditions and oral squamous cell carcinoma. As far as our knowledge furthermore research work is required to confirm the previously obtained results.

COX 2 & ORAL CANCER

COX-2 is an inducible form of cyclooxygenase that is over-expressed during inflammation and can lead to carcinogenesis. In most normal tissues, the expression of pro-inflammatory genes like COX-2 is hardly detectable and induced in response to pro-inflammatory stimuli such as bacterial wall protein LPS.[23] The LPS stimulated cells can secrete pro-inflammatory mediators like prostaglandins.[24]

Literature review indicates the initial role of COX-2 in carcinogenesis as a well-established tumor promoter.[25] Many tumors secrete high levels of pro-inflammatory cytokines, including PGE2[26] and thus can induce pro-inflammatory microenvironments. PGE2 promotes cell proliferation and favours tumor growth by inhibiting cell death.[25]

PGE2 in the tumour microenvironment has the capacity to suppress antitumour immune responses, firstly by altering the functions of dendritic cells, resulting in suboptimal generation of antitumour-specific cytotoxic T cell activation; and secondly by promoting differentiation of immunosuppressive regulatory T cells that downregulate antitumour immune responses[27] through TGF- β and IL-10.[28] On the other hand, some immunoinflammatory responses, particularly Th1 and possibly Th17 responses within the tumour-associated stroma represent anti-tumour protective reactions aimed at eradicating tumour cells by cytotoxic T lymphocytes (CTL) and natural killer cells.[29]

Chan et al. is the first one to report the up-regulation of COX-2 in head and neck squamous cell carcinoma (HNSCC). Head and neck squamous cell carcinoma (HNSCC) tissues

show a higher level of COX-2 protein and 150-fold greater Cox-2 mRNA expression than healthy oral mucosa.[30] COX-2 plays a vital role in mediating the inflammatory process. COX-1 is constitutive isoenzyme that regulates homeostasis by maintaining the physiological level of prostaglandins whereas COX-2 is inducible and up-regulated by a number of stimuli such as cytokines, mitogens, oncogenes, growth factor and tumor promoters. Altered expression levels of other cellular markers, such as proliferating cell nuclear antigen, cytokeratins, enzymes (COX-2), antiapoptotic genes (*bcl* family), proangiogenic genes (VEGF family), and immunomodulators (IL-10 and IL-12), have been implicated in squamous cell carcinoma progression.[30–35]

Extra production of PGE₂ and increased COX-2 activity are oftenly observed in a variety of malignancies including breast, prostate, bladder, liver, pancreas, skin, lung, colon and brain. Therefore, prostaglandin synthesis suppression through the selective inhibition of COX-2 is now regarded as a new practical approach to cancer prevention.[36,37] Hence, it is not surprising that in present era much attention has been focused on antigenotoxic/antioxidant effects and COX-2 inhibitory activity of natural phytochemicals from plants.

Cyclooxygenase-2 (COX-2) has been known to act as a survival factor in a variety of cellular stress conditions and to protect normal human cells such as neurons, cardiomyocytes, renal cells, mammary epithelial cells, fibroblasts and endothelial cells from apoptosis induced by various stresses including nerve growth factor withdrawal, ischemia, hypertonicity or DNA-damaging agents.[38] However, COX-2 is also considered a pro-carcinogen as demonstrated by experiments where tumorigenesis was inhibited in COX-2 knockout mice and by cancer chemoprevention studies, which used nonsteroidal anti-inflammatory drugs.[39]

COX-2, an enzyme that catalyzes the synthesis of prostaglandins, is overexpressed in a variety of premalignant and malignant conditions, including oral leukoplakia and squamous cell carcinoma of the head and neck.[40] The increase of COX-2 enzyme may contribute to carcinogenesis by modulating xenobiotic metabolism, apoptosis, immune surveillance, and angiogenesis. Recent evidence by Tsujii et al.[31] and Masunaga et al.[41] has shown that COX-2 augments the release of angiogenic peptides as a result of increased production of its metabolite PGE₂, and a positive correlation of COX-2 versus microvessel density was observed.

The ingredients of betel quid have been shown to up-regulate prostaglandin production, cyclooxygenase-2 mRNA and protein expression of human oral keratinocytes.[42] COX-2 overexpression has been found in polyps and cancer of the colon, as well as many neoplastic cells, including those associated with oral cancer [43], and has been proposed as a cancer treatment target.

COX-2 is quickly induced by factors that are implicated in carcinogenesis, for example, growth factors, inflammatory stimuli, oncogenes, and tumor promoters. COX-2 deletion in *Apck* knockout mice greatly reduces intestinal polyp formation, providing genetic evidence that COX-2 plays a role in tumorigenesis.

The relationship between COX-2 expression and tumor differentiation in SCCs is still controversial. In the lung [44], esophagus [45] and larynx [46], it was reported that COX-2 expression was elevated in well-differentiated carcinomas more than in poorly differentiated carcinomas. On the other hand, it was reported in SCCs of the tongue [47] and of the esophagus [48] that more undifferentiated carcinoma (histological grade III) had significantly stronger COX-2 expression than grade I or II cases.

By reviewing the literature, we found a report on hepatocyte growth factor inhibiting anoikis by induction of COX-2 in human head and neck cancer UMSCC1 cells, the mechanism of which remains to be explained.[49] COX-2 also inhibits anoikis by activation of the PI-3K/Akt pathway in human bladder cancer EJ cells, which provides one of the mechanisms for the anti-anoikis effect of COX-2.[50] A recent *in vitro* study also demonstrated that wild-type p53 inhibits the formation of the complex between TBP and human COX-2 promoters in a cell free system.[51] In addition, COX-2 has been known to inhibit apoptosis in human cancer cells through the regulation of Bcl-2 family protein expression.[52–54]

Accumulating evidence indicates that COX-2 plays an important role in tumor development and progression. In several epidemiologic studies, regular administration of COX-2 promotes colon cancer development [55,56] Various combinations of surgical resection, radiation, and/or chemotherapy, are the most effective treatments of cancer if cancer is diagnosed at an early stage. But it is very difficult to identify all individuals who are at the highest risk for developing cancer may be due to irresponsibility of individuals who report to the hospital when the disease becomes severe, lack of readily available advanced diagnostic modalities in every health sector. There is reported lower five year survival rate for the cancer patients as they present to the hospital with more advanced stage of cancer hence these standard treatment regimens fail to completely cure the disease. It is generally agreed that an effective way to control cancer is to find better ways of preventing it. Chemopreventive approaches are definitely worth considering for healthy persons who have a strong family history of cancer or those who are particularly susceptible for other reasons. One promising group of compounds with cancer preventive activity includes NSAIDs. Accumulating evidence also indicates that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the occurrence or progression of colorectal cancer and polyps.[59,60] COX-2 selective inhibitors are actively being tested in clinical trials for the prevention of several cancers including, colorectal, esophageal adenocarcinoma, and head and neck cancer.

The computer-aided drug design approaches were employed to develop potent COX-2 inhibitors, that are attractive and potential leads for various human cancer and inflammatory disorders. The crystal structure of COX-2 could be useful to provide a better understanding about the active sites as well as about the protein-inhibitor binding mechanism.[61] However, how and why the COX-2 gene is constitutively and highly expressed in tumor cells has not yet been clarified.

Although surgical resection is the first choice for oral cancer, the development of new anti-cancer drugs is of great interest. An *in vitro* study was conducted by Yukako Mikiet.al. to evaluate the effect of the histone deacetylation inhibitor, sodium butyrate (NaBu) on oral cancer cell (OCC) HSC-3 and HSC-4 proliferation. They have examined the

synthesis of rate-limiting enzymes such as sPLA2 (-IIA, -V, -X) and COX- 2 by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot, as well as PGE2 by ELISA in their study. They have found that NaBu inhibits oral cancer cell proliferation in a concentration-dependent manner. The effects of NaBu clearly demonstrated an association with cell cycle arrest and increased expression of p21^{Cip1}/WAF1. NaBu also regulated COX-2 and sPLA2-X gene transcription. COX-2 has recently become a focus for cancer chemoprevention.[59]

A study conducted by Tsujii et.al. have shown a positive correlation between the expression of COX-2 and inhibition of apoptosis.[32] Danial and Korsmeyer stated that cell apoptosis is characterized by distinct morphological changes, such as plasma membrane blebbing, cell shrinkage, mitochondrial depolarization, chromatin condensation and DNA fragmentation.[62]

There have been a few studies evaluating the effects of single-nucleotide polymorphisms (SNPs) on the predisposition of PMDs. SNPs of the COX-2 gene also have been associated with the etiology of a wide variety of solid tumors, including oral squamous cell carcinoma.[60,63,64] Recently in the literature it's been observed that the exon 10 p837 SNP, a potentially functional SNP located in the 3' untranslated region (UTR) of the COX-2 gene, may modulate the risk of bladder cancer through regulating the steady-state messenger RNA (mRNA) expression of COX-2.[65] Moreover, Lin et al reported that a COX-2 promoter variant, - 765 G C, exhibited distinct effects on the development of different subtypes of oral cancer and PMDs.[63]

Xia Puet.al.have assessed the associations between multiple functional variants of the COX-2 gene and susceptibility to PMDs. They have conducted a case-control analysis to evaluate& assess the individual and haplotype/diplotype effects of the 3 most studied potentially functional polymorphisms of the COX-2 gene on PMD predisposition and have found that the exon 10 + 837T C SNP is located in the 3' -UTR region, which may play an important role in the regulation of mRNA stability and translation.[66]

One of the important hallmarks for cancer progression is DNA damage, resulting either from various carcinogens accumulating from etiologic influences or due to genetic errors.[67] If detectable and quantifiable, these may contribute towards an easy detection and prediction system for oral cancer development and prognosis.

Several chronic human diseases associated with inflammation are characterized by over production of reactive oxygen species (ROS).[68] ROS production plays an important role in the modulation of inflammatory reactions.ROS damage involves single or double stranded DNA breaks, purine and pyrimidine modifications, DNA intrastrand adducts and DNA crosslinks. Increase in oxidative stress overwhelms repair systems and lead to cellular damage. Direct damage to DNA by ROS contributes to the development of cancer. Several evidences suggest a link between oxidative stress, cyclooxygenases and cancer.[69]

No reliable prognostic marker for risk assessment in putatively pre-malignant disorders of oral cavities has emerged until recently. COX-2 was recently proposed to be a diagnostic marker for early detection of the malignancy.[70]

CONCLUSION

Inflammation is a recently defined contributor of oral carcinogenesis. In this multi-step process, inflammation might have a role in initiation as well as progression. Since persistent inflammation promotes cell proliferation and may induce DNA damage; and that at initial stages of tumorigenesis, inflammatory factors mediate the development of a tumour associated stroma. In turn, activated cells in this stroma promote angiogenesis, cancer growth and metastasis, and mediate immune evasion. Important inflammatory components are cytokines and chemokines produced by activated innate immune cells, and cox 2 has found to be one among them, which stimulate tumor growth and progression. Moreover, genetic susceptibility and gene/environment interactions are becoming more important in the attempt to eliminate the burden of cancer. The evidence found so far is sending out signals that OSCC may cease to exist in the future, and the referral will only be for a group of diseases that manifests symptoms of a similar sort. Further studies with larger sample groups in premalignant diseases of oral mucosa as well as OSCC are required to confirm these findings.

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