Cyclooxygenase-2 in Oral Cancer: A Review

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ABSTRACT
Cyclooxygenase (COX)-2 is a rate-limiting enzyme in the conversion of arachidonic acid to prostaglandin. It is up-regulated in inflammation as well as in various cancer tissues like colon, stomach, breast, lung and head and neck including oral cavity. Overexpression of COX-2 inhibits apoptosis and immune surveillance; promote angiogenesis, increases cancer invasiveness and metastasis. Therefore, COX-2 is considered to be strongly involved in carcinogenesis and tumor growth. Immunohistochemical and Western blot analyses in oral precancerous and cancerous lesions demonstrated that COX-2 expression is increased from epithelial dysplasia to squamous cell carcinoma, with the elevation of cell proliferating activity. Inhibition of COX-2 activity with selective COX-2 inhibitors results in suppression of tumor cell growth and invasion in vitro. Thus, these evidences indicate that COX-2 becomes a potent molecular target for prevention and therapy of oral cancer.

KEYWORDS: Oral squamous cell carcinoma, Cyclooxygenase-2, cox-2 inhibitor.

Introduction
Cyclooxygenase (Cox) is an enzyme that plays a crucial role in the synthesis of prostaglandins (PG) from arachidonic acid (AA). This enzyme is liberated from membrane glycerophospholipids by the phospholipase (PLA) family of enzymes. Prostaglandin E2, one of the major products of this conversation, is a major mediator of inflammation and angiogenesis. Cox is present in two isoforms i.e. Cyclooxygenase-1 (cox1) and Cyclooxygenase-2 (cox2). The Cox-2 is also known as a prostaglandin-endoperoxidase synthase 2 (PTGS2), prostaglandin synthase (PG synthase) in humans. A third form (cox3) has been recently identified. Dan Simmon's group demonstrated the existence of this new Cox stating it as a splice variant of cox1 and found more abundant in cerebral cortex and heart.

Under normal circumstances COX-2 is usually not expressed in most cells, while increased levels are found in response to inflammation, growth factors, and tumor promoters.

Cox-2 being an inducible enzyme gets induced by a variety of pathophysiological conditions of tissues by growth factors, inflammatory stimuli, oncogenes and tumor promoters. Cox-2 may play a role in the inhibition of apoptosis, immune surveillance, promotion of angiogenesis, promotion of cancer invasiveness and metastasis. It has also been known to modulate cell differentiation. Cox-2 induced PGs play a role in the accelerated cellular proliferation causing malignant transformation. Thus, suggesting its role where it was found to be up-regulated in most of the studies carried out in oral squamous cell carcinoma (OSCC).

Discussion
Cox is an integral membrane protein found mainly in microsomal membranes. In the early 1990's it was established that there are at least two Cox isoforms present in human cells. They are the products of two distinct genes localized on chromosome 9 and 1, respectively. The cox1 gene is about 22kb in length and cox2 is 8kb long with 10 exons. Confocal fluorescence imaging microscopy and histofluorescence techniques reveal that both Cyclooxygenases are largely located on the luminal side of the endoplasmic reticulum (ER) membrane and the nucleus envelop, although they have also been detected in some situations in lipid bodies, mitochondria, filamentous structure vesicles and in the nucleus. Cox1 is universally expressed in mammalian tissues and cells, whereas cox2 is generally present at very low levels and increased on stimuli such as cytokines and growth factors and therefore it is referred to as the inducible Cox isoforms.

Cox1 and cox2 differ mainly in their amino acid arrangement i.e. 17 amino acid sequence near its amino terminus in former while 18 near carboxyl terminus in later. The differential regulation of expression and tissue distribution being the most striking distinction.

The Cyclooxygenase enzyme catalyzes the rate limiting step in conversion of AA to prostaglandin G2 (PG-G2), further reduction to PGH2 later on being converted to structurally related PGs by PG synthase.

Cox2 is known to express in as an inflammatory response in humans. Inflammation is a non-specific immune response to infection, irritation or other injury; it is characterized by redness, swelling, pain and loss of function. When harmful agents invade the human body, inflammatory mediators are released by immune cells, this release causes vasodilatation, migration of neutrophils, chemotaxis and increased vascular permeability. Two separate pathways produce...
inflammatory mediators namely cyclooxydation and peroxidation.\textsuperscript{1}

Carcinogenesis and cancer progression associated with chronic inflammation is mediated by cox-2 which is caused by various factors such as bacterial infections and chemical irritants. The risk of carcinogenesis increases with the longer duration of associated inflammation. Although neoplasia is caused by various inflammatory mediators that induces preneoplastic mutation, cell proliferation, invasion and metastasis, stimulation of angiogenesis and resistance to apoptosis, these inflammatory mediators then activate signaling molecules involved in inflammation and carcinogenesis such as cox-2 and nuclear factor-kappa B (NF-kB).\textsuperscript{12}

Cox is the key-enzyme catalyzing the first step in the biosynthesis of prostaglandins. In particular cox-2 act synergistically with cytosolic phospholipase A2 participating in the process of carcinogenesis and both enzymes are overexpressed in dysplasia and carcinoma.\textsuperscript{2}

Overexpression of COX-2 is reported in many cancers due to its involvement in several key-mechanisms like 1) the conversion of pro-carcinogens to carcinogens as a consequence of arachidonic acid metabolism, 2) stimulation of cell growth, 3) inhibition of apoptosis through P53 suppression and Bcl2 induction, 4) stimulation of VEGF and angiogenesis, 5) promotion of invasion and metastasis via matrix metalloproteinases (MMP) induction, 6) immunosuppress ion by IL-10 induction.

Recent studies have shown that up-regulation of cox2 is found in various types of human carcinomas, \textsuperscript{11, 12} including intestinal adenocarcinomas,\textsuperscript{4} lung carcinoma,\textsuperscript{11} and squamous cell carcinoma of the head and neck.\textsuperscript{11,12}

Hida T et al\textsuperscript{13} in 1998 immunohistochemically examined cox2 expression in 59 human lung cancers as well as in normal and premalignant lung specimens. Up-regulated COX-2 expression was detected in about one-third of atypical adenomatus hyperplasia and carcinoma in situ specimens, and a significant increase in COX-2 expression was observed in 70\% of invasive adenocarcinoma cases suggesting that an increase in COX-2 expression may be associated with the development of adenocarcinoma.

Huang M et al\textsuperscript{14} in 1998 obtained resected specimens of non-small cell lung cancer (NSCLC) and revealed cytoplasmic staining for cox2 within tumor cells immunohistochemically.

Wolff H et al\textsuperscript{15} in 1998 studied the expression of Cox-2 mRNA and protein in human lung adenocarcinoma, squamous cell carcinoma, and small cell lung cancer. Cox-2 mRNA levels were high in well-differentiated adenocarcinoma samples, but low in poorly differentiated adenocarcinoma, squamous cell carcinoma, and small cell lung cancer, as detected by Northern blot analysis. Immunohistochemy showed Cox-2 staining in 19 out of 21 adenocarcinomas. However, well-differentiated adenocarcinoma contained more Cox-2 staining than the poorly differentiated ones.

Tomozowa S et al\textsuperscript{16} in 2000 studied cox2 expression in advanced human colorectal cancer and its correlation with clinicopathological features using IHC and observed cox2 positivity mainly in cytoplasm of cancer cells in all the specimens.

Cox2 overexpression was also noted in gastric\textsuperscript{17}, cervical\textsuperscript{18}, prostate\textsuperscript{19}, breast\textsuperscript{20}, esophageal\textsuperscript{21}, endometrial\textsuperscript{22}, and skin\textsuperscript{23} and gallbladder\textsuperscript{24} cancers.

Saukkonen K et al\textsuperscript{20} 2001 examined COX2 expression in human gastric dysplasia and adenocarcinoma and found 58\% positivity in well-differentiated while 6\% in poorly differentiated type. When examination carried out on the cell lines also showed the same results i.e. higher expression of COX2 mRNA, protein and enzymatic activity in well-differentiated compared to poorly differentiated. Additionally 44\% of dysplasia with no evidence of invasion showed COX2 positivity.

Ristimaki A et al\textsuperscript{21} analyzed the expression of cox2 proteins by IHC in tissue array specimens of 1576 invasive breast carcinomas and observed elevated expression of cox2 protein in 37.4\% of the tumors. It was associated with unfavorable distant disease–free survival indicating cox2 expression is more common in breast cancers with poor prognostic characteristics and is associated with an unfavorable outcome.

Zhi H et al\textsuperscript{22} in 2006 determined the pattern of COX2 expression in normal esophageal mucosa, dysplasia, carcinoma in situ (CIS) and invasive SCC. Immunohistochemical analysis showed COX2 was weakly expressed in normal squamous epithelium, strongly in early stage of dysplasia (77\%) and in invasive SCC (77\%).

Jarzabek K et al\textsuperscript{23} 2013 evaluated cox-2 expression at the mRNA and protein levels using quantitative real-time PCR and IHC method in 51 endometrial carcinoma and 16 normal endometrial. The majority of tumors expressed cox2 (88\%) proteins.

There is strong evidence from experiments in animal models and epidemiologic studies that Cyclooxygenases are involved in the promotion and progression stages of non-melanoma skin cancer (NMSC), and therefore, are excellent targets for the prevention of NMSCs.\textsuperscript{44}

Legan M et al\textsuperscript{24} in 2006 examined the relationship between COX2 overexpression and p53 accumulation in gallbladder carcinoma and its precursor lesions showing COX2 expression in 14.3\% of normal gallbladder epithelium, 70.3\% of dysplastic epithelium, and 59.2\% of adenocarcinoma. P53 showed 31.2\% positivity in high-grade dysplasia and 48.1\% in carcinoma. They also observed a significant difference in COX2 expression among normal epithelium, low-grade dysplasia and high-grade dysplasia suggesting that overexpression of COX2 is an early event in gallbladder carcinogenesis.

Oral cancer is the eighth most common head and neck cancer worldwide with high morbidity and mortality, affecting 6,00,000 new patients each year.\textsuperscript{25} In India, OSCC is the leading cancer in men and fifth common cancer in women.\textsuperscript{26} Though oral cavity is readily accessible for clinical examination, most tumors are not diagnosed until they have advanced or metastasized.44 Furthermore, the majority of oral cancer patients had a significantly poor prognosis and the overall five year survival rate of oral cancer patients is less than 50\%.\textsuperscript{27}

The etiology of oral cancer is multifactorial and includes genetic components, environmental components, viral infections and social and behavioral factors.\textsuperscript{25} the underlying mechanisms appear to converge on inflammation related pathways. Inflammation is closely related to altered gene expression of oncogenes and tumor suppressor genes and is a major factor in promoting neoplastic transformation.\textsuperscript{25}

Chan G et al\textsuperscript{28} in 1999 studied the cox2 expression in squamous cell carcinoma of the head and neck (HNSCC) by quantitative reverse transcription-PCR (qPCR), immunoblotting and immunohistoche mistry and found it to be overexpressed in all the cases and in all the methods thus, concluding cox2 is up-regulated in HNSCC.

Itoh S et al\textsuperscript{29} 2003 investigated COX2 expression in 72 OSCC samples by IHC, and correlated its expression with clinicopathologic variables. Cancer cells at invasive front showed strong positivity. The dysplastic basal cells at the margins of the tumor were also COX2 positive which concludes, the evidence of COX2 in OSCC and that it
can be a useful predictor for prognosis.

In 2003, the immunohistochemical findings verified by western blotting showed up-regulated cox2 from healthy to premalignant to cancerous (88%) oral mucosa.27

Shibata M et al24 in 2005 correlated the Cox expression with pathological nature of normal oral mucosa, dysplasia and OSCC and found out cox2 being highest in severe dysplasia, significant inverse correlation with the histological differentiation of OSCC. The expression of cox2 in the blood vessels of esophageal cancer suggested that cox2 may promote angiogenesis and lack of cox2 may prevent new blood vessel formation in esophageal epithelium.

Pandey M et al25 in 2008 evaluated cox2 gene expression using reverse transcriptase polymerase chain reaction (RT-PCR) which showed up-regulation in oral cancer and pre-cancer suggesting a role for cox2 receptors in oral carcinogenesis.

Saba NF et al21 in 2009 studied cox2 expression by IHC in normal tissues, premalignant lesions, and carcinoma in situ (CIS). They also correlated cox2 expression with clinical characteristics in HNSCC. Cox2 was expressed in early and intermediate stages of premalignant lesions, increasing in the basal and parabasal layers. It was also noted to increase in severe/CIS stage and invasive carcinoma.

Mauro et al22 in 2010 investigated cox2 expression in normal human oral mucosa, hyperplasia, dysplasia and carcinoma by IHC and RT-PCR. Results showed cox2 is not expressed in the normal tissue, starts to express in hyperplasia, reaches the maximum activation in dysplasia and then starts to down-regulate in carcinoma.

Ryott M et al23 in 2011 examined the prognostic value of cox2 expression in 76 samples in oral tongue squamous cell carcinoma (OTSCC) between January 2000 and December 2004 by IHC. All OTSCC specimen expressed cox2. Its staining intensity increased significantly with more advanced stage. 50% of the surgical specimens showed a decrease in immunostaining post-radiation. No association was found with survival suggesting cox2 expression has limiting prognostic value in OTSCC.

Li Tj et al24 in 2013 observed cox2 expression in normal oral mucosa, oral lichen planus (OLP) and OSCC by IHC and RT-PCR. They concluded the abnormal expression of cox2 and MMP7 are closely related to the biological behavior of OSCC. The MMP7 may be induced by cox2 further leading to invasion and metastasis.

Pontes HA et al25 in 2013 verified the immunoexpression of cox2 proteins in dysplastic oral lesions and oral squamous cell carcinoma and confirmed the early involvement of cox2 in oral carcinogenesis.

Wang ZM et al26 in 2014 performed a meta-analysis to identify impact of cox2 expression on prognosis in oral cancer. Results showed significant correlation between cox2 protein expression and the recurrence-free or overall survival rate of oral cancer, suggesting cox2 may have a prognostic significance in oral cancer.

Li D et al27 in 2015 investigated the association between functional cox2 gene polymorphism and the risk of oral cancer by meta-analysis. The results revealed a strong association between the polymorphisms and the susceptibility to oral cancer. Therefore, cox2 polymorphisms are linked to increased risk of oral cancers.

Silva LP et al28 in 2017 determined the IHC expression of cox2 in OSCC and its association with histological grades of malignancy and metastasis and found out higher expression of cox2 in tumors of low-grade malignancy.

Cancer and Cox inhibitor

There is increasing evidence that cox2 inhibitors could play a role in chemoprevention of epithelial cancer. The hypothesis of Cox involvement in cancer progression has been strengthened by the effect of Cox inhibitors that have been successfully used in the treatment of many cancers. The efficacy of Cox selective and nonselective inhibitors against oral carcinogenesis has been confirmed by in vitro and in vivo studies on rat25 Cox inhibitors showed to be chemopreventive decreasing oral cancer incidence, cell growth, proliferation of xenografted tumors, cancer invasion score and cancer-related mortality.

The impact of cox2 inhibition was assessed in a SCC cell line (NS-398) and inhibition of proliferation of cancer cells expressing cox2 mRNA was seen and attributed to suppression of PGE2 production.29

It is observed in patients who use extensive amounts of non-steroidal anti-inflammatory drugs (NSAIDs) showed lower cancer incidence. This antineoplastic effect of NSAIDs is supported by both observational and controlled epidemiological studies that have shown that prolonged use of aspirin and other NSAIDs is associated with 40-50% reduction in the risk of colorectal cancer30 as well as esophageal cancer.

Milas et al31 in 1990 described that NSAIDs and selective COX-2 inhibitors increased tumor radioresponse by restoring im munoreactivity.

Gorski et al32 in 1999 proposed that the anti-angiogenic actions of COX-2 inhibitors produced radiodensitisation via blockade of the VEGF stress response.

Amirghahari et al33 in 2003 reported that NS-398 demonstrated the radiosensitising effect on human HNSCC cell line HEPd via inhibition of radiation-induced up-regulation of COX-2 protein expression.

Raju et al34 in 2005 reported that celecoxib strongly enhanced the sensitivity of human head and neck cancer cell line, HN-5, to radiation via down-regulation of the expression of Ku70 protein and inhibition of the kinase activity of DNA-PKs which are involved in the double-stranded DNA-break repair.

In inoperable/unresectable non-small lung cancer, a phase I clinical trial of thoracic radiotherapy and concurrent celecoxib has already been performed, and has proven the safety of celecoxib administration and an encouraging outcome of local progression-free survival.

Rathore K et al35 in 2014 evaluated the effects of novel receptor tyrosine kinase inhibitor and NSAID piroxicam on the OSCC cell lines (FaOSCC-Sidney and K9OSCC-Abby) and found out piroxicam inhibited the cox-2 expression in all tested OSCCs.

Treatment with a Cox inhibitor reduces cox2 mRNA, cox2 protein and PGE2 synthesis in mammary and oral epithelial cells. The mechanism of action of the cox2 inhibitor was shown to involve inhibition of protein kinase C, extracellular signal-regulated kinase and p38 mitogen-activated protein kinases.

Another potent therapy is the usage of cox-2 inhibitors in combination with chemotherapy. Cox-2 inhibitors have been shown to potentiate the cytotoxic action of chemotherapeutic agents in vitro and to improve their anti-tumor efficacy in vivo. Hida et al36 in 1998 reported that nimesulide induce apoptosis and enhanced cytoxicity of cisplatin and etoposide (VP-16) in lung cancer cells.

Duffy et al37 in 1998 reported that cox-2 inhibitors showed a concentration dependant synergistic anti-tumor effect on human lung cancer cell lines and a human leukemia line in combination with anthracycline, teniposide, etoposide and vincristine.

Hashitani Set al38 in 2003 reported that combination of celecoxib with anti-cancer drugs, particularly doxorubicin, vincristine and bleomycin, enhanced the cytotoxicity against human head and
neck carcinoma cell lines by increased induction of apoptosis.

In vivo studies, Hida et al in 2002 reported that combined use of JTE-522 and conventional anti-cancer agents such as doxetaxel and vinorelbine significantly increased the efficacy of both in vitro and in vitro growth inhibition in human lung cancer cells.

Nakata et al in 2004 demonstrated that celecoxib enhanced the anti-tumor efficacy of doxetaxel and radiation on human A431 tumor xenografts in mice and that the greatest effect was achieved when all three agents were combined. They suggested that this improvement of anti-tumor efficacy is attributed to the augmentation of apoptotic cell death.

In patients with early-stage non-small cell lung cancer, Altorsi et al in 2009 found that the addition of celecoxib enhanced the response to preoperative paclitaxel and carboplatin.

Therefore, the combination of COX-2 inhibitors with chemotherapeutic agents and/or irradiation is considered to have a high potential for oral cancer treatment.

**Conclusion**

COX-2 is considered to be strongly involved in carcinogenesis and tumor growth. The patients with overexpression of COX-2 showed poor prognosis and their overall 5-year survival rate was decreased. Inhibition of COX-2 activity with selective COX-2 inhibitors results in suppression of tumor cell growth and invasion in vitro and prevention of oral carcinogenesis by chemical carcinogens in animal models. Also, combined use of selective COX-2 inhibitors was found to enhance synergistically the cytotoxic effects of anti-cancer drugs and irradiation. From these evidences, it is indicated that COX-2 becomes a potent molecular target for prevention and therapy of oral cancer.

**References**


