Review Article

Targeted Therapy of Oral Cancer: a Focus on COX-2 Inhibitors

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Abstract

Oral squamous cell carcinoma (OSCC) is one of the major leading cancers worldwide. Generally, the incidence of OSCC remains constant globally but appears increasing in some parts of the world especially in the young group. Despite an easy access to early diagnosis and treatment, the mortality rate of patients with OSCC remains high due to delayed treatment and failure to control tumor recurrences and metastasis. The overall five-year survival rate for OSCC is considerably lower than other cancers and has not significantly changed during the last two decades. Recently, novel treatments, aiming to target specific molecules, aberrantly expressed during OSCC carcinogenesis, have been investigated and tested in clinical trials at several research settings. COX-2, in particular, is significantly overexpressed in OSCC and suggested to play a crucial role in OSCC carcinogenesis. This review will focus on the association between OSCC and COX-2 and the use of the COX-2 inhibitors as the targeted therapy for OSCC. This new approach in OSCC treatment offers hope that it may replace the conventional treatment modalities considered nonspecific and causing unwanted severe complications to patients.

Key words : COX-2 Inhibitors; Oral squamous cell carcinoma; Targeted therapy

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Introduction

Head and neck cancer is the sixth most common cancer worldwide.[1] More than 90 % of head and neck cancers are of squamous cell carcinoma type. Head and neck cancer includes cancers primarily located in the oral cavity, oropharynx, nasopharynx, hypopharynx and larynx.[2] It is estimated that globally 650,000 patients are annually diagnosed with head and neck squamous cell carcinoma (HNSCC).[2] In this review, the author will focus mainly on oral squamous cell carcinoma (OSCC), the most common type of HNSCC, and targeted therapy for OSCC especially by the use of the COX-2 inhibitors.

Oral squamous cell carcinoma is a common cancer worldwide including Thailand where there is a high occurrence rate in young people.[3] OSCC is aggressive and causes high morbidity and mortality in patients. In general, men are affected more frequently than women because of the more risky habits in men such as tobacco use and alcohol consumption.[4] However, an increase of these risk habits in females has resulted in more female patients with OSCC. In South, Southeast Asian and West Pacific countries, betel quid chewing habit remains a prominent risk for OSCC.[5] Nevertheless, this habit appears to diminish in the younger population of the northern part of Thailand.[4] Recent data have shown that human papillomaviruses (HPV) particularly HPV types 16 and 18 are regarded as an emerging risk for OSCC.[6] Notably, HPV-related HNSCC shows a distinctive clinical course and better clinical outcomes[7] suggesting a necessity to assess risk factors in each patient with OSCC. Other risk factors for OSCC include low intake of fresh fruit and vegetable, low socio-economic status and immunosuppression.[8] Currently, our group is assessing the risk factors of OSCC in Thailand aiming at preventing OSCC by minimizing those risks in a population at large.

In terms of treatment, surgical resection remains the primary modality for OSCC.[9] A combined surgery and radiation modality is used in patients with more advanced stages. For patients with unresectable disease, concurrent cisplatin-based chemoradiation (CRT) is recommended as this treatment modality leads to better overall five-year survival and locoregional control.[10] Recently, many investigators have attempted to target specific molecules or proteins that cancer cells utilize for their growth and progression. It is hopeful that the targeted therapy can eventually replace the conventional treatment modality by inhibiting tumor growth, invasion and metastasis resulting in prolonging patients' survival. In OSCC, many studies have found an overexpression of cyclooxygenase-2 (COX-2), an inducible enzyme that catalyzes the synthesis of prostaglandins from arachidonic acid (AA).[11,12] In addition, epidermal growth factor receptor (EGFR) and its downstream signaling molecules such as Akt are overexpressed in OSCC.[13,14] As a result of overexpression of COX-2 and Akt, cancer cells are proliferating, resistant to apoptosis, and switched on to the epithelial-mesenchymal transition (EMT), leading to tumor invasion and metastasis.[15] Therefore, targeting COX-2 and

perhaps together with molecules involving in the EGF signaling pathway and its receptor should be an effective approach for controlling growth, invasion, and metastasis of OSCC.

Cyclooxygenase

Cyclooxygenase or prostaglandin-endoperoxide synthase, is an enzyme responsible for the conversion of AA into various prostaglandins (PG_s).[16,17] Two COX isoforms have been discovered: COX-1 and COX-2. Although COX-1 and COX-2 share a high level of homology, the activity and expression of these enzymes are different, and they can actually function independently within the same cell type. The *COX-1* gene is a type of housekeeping gene and expressed constitutively in a wide range of tissues such as the lung, kidney, stomach and intestines. The basic functions of COX-1 are not only promoting the synthesis of PG_s, but also maintaining the homeostasis of an organism such as regulating the clotting mechanism, stabilizing renal blood flow and protecting gastric mucosa.[18] COX-1 is expressed negatively or only weakly in tumor cells and not involved in carcinogenesis. The *COX-2* gene is expressed negatively in normal tissues and organs under physiological conditions, except constitutive expression in the kidney, seminal vesicles and brain.[17] It is inducible in response to certain stimuli such as carcinogens, cancer-causing phorbol esters, oncoproteins, growth factors and cytokines.[16,17,19] In response to pro-inflammatory cytokines, AA is converted to many types of PGs and thromboxanes (TXs), leading to inflammatory reactions and increased pain responses in patients (Fig. 1). Moreover, COX-2-derived PGs can stimulate cell proliferation, promote angiogenesis, increase invasiveness and adhesion to the extracellular matrix and inhibit immune surveillance and apoptosis in cancers.[16,19]

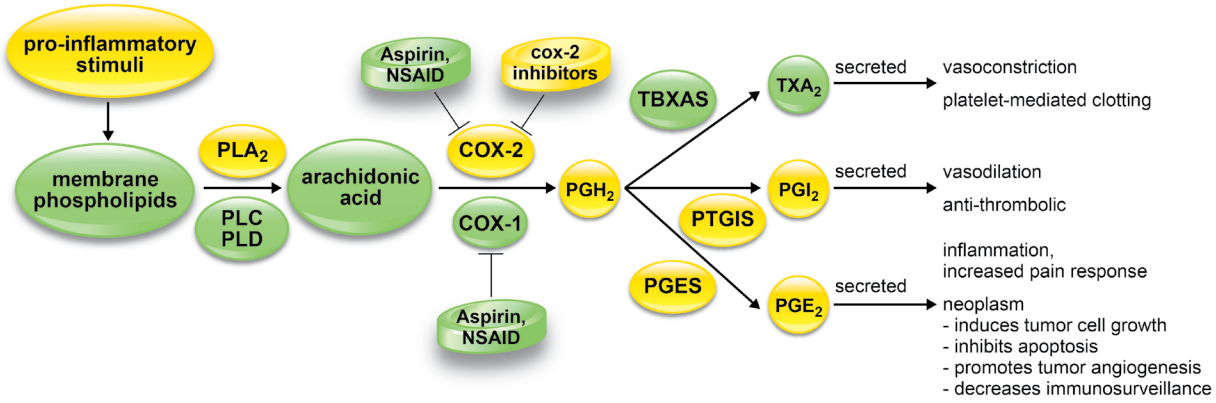

Figure 1 *The COX-2/PGE2 pathway. Upon induced by certain pro-inflammatory stimuli, membrane phospholipids are converted to arachidonic acid (AA) by phospholipase (PL) enzymes. Subsequently, cyclooxygenase-2 (COX-2) catalyzes AA to many types of prostaglandins (PGs) and thromboxanes (TXs), leading to inflammatory responses and carcinogenesis in patients. COX-2 inhibitors, in particular, can selectively block the formation of PGs, resulting in decreased inflammatory responses and carcinogenesis (adapted from Stasinopoulos et al.).*[16]

Role of COX-2 and inflammation in cancers

It has long been known that chronic inflammation, caused by a variety of factors, including bacterial, fungal, viral, parasitic infections, chemical irritants and nondigestible particles can predispose individuals to cancers.[20] The longer the inflammation persists, the higher the risk of associated carcinogenesis. Two pathways, intrinsic and extrinsic pathways, are postulated as links between inflammation and carcinogenesis.[21,22] The intrinsic pathway is suggested to be driven by genetic events that cause carcinogenesis, while the extrinsic pathway is driven by inflammatory conditions predisposing to cancer (Fig. 2). Recent findings have shown that chronic inflammation probably via some inflammatory mediators such as COX-2 and nuclear factor-kappa B (NF-κB) can promote all stages of tumorigenesis, including DNA damage, limitless replication, apoptosis evasion, sustained angiogenesis, self-sufficiency in growth signaling, insensitivity to anti-growth signaling, and tissue invasion and metastasis.[17] Various inflammation networks are thought to play a vital role in the microenvironment of cancer tissues and one of the most important networks appears to be the COX-2/PGE_2 pathway.

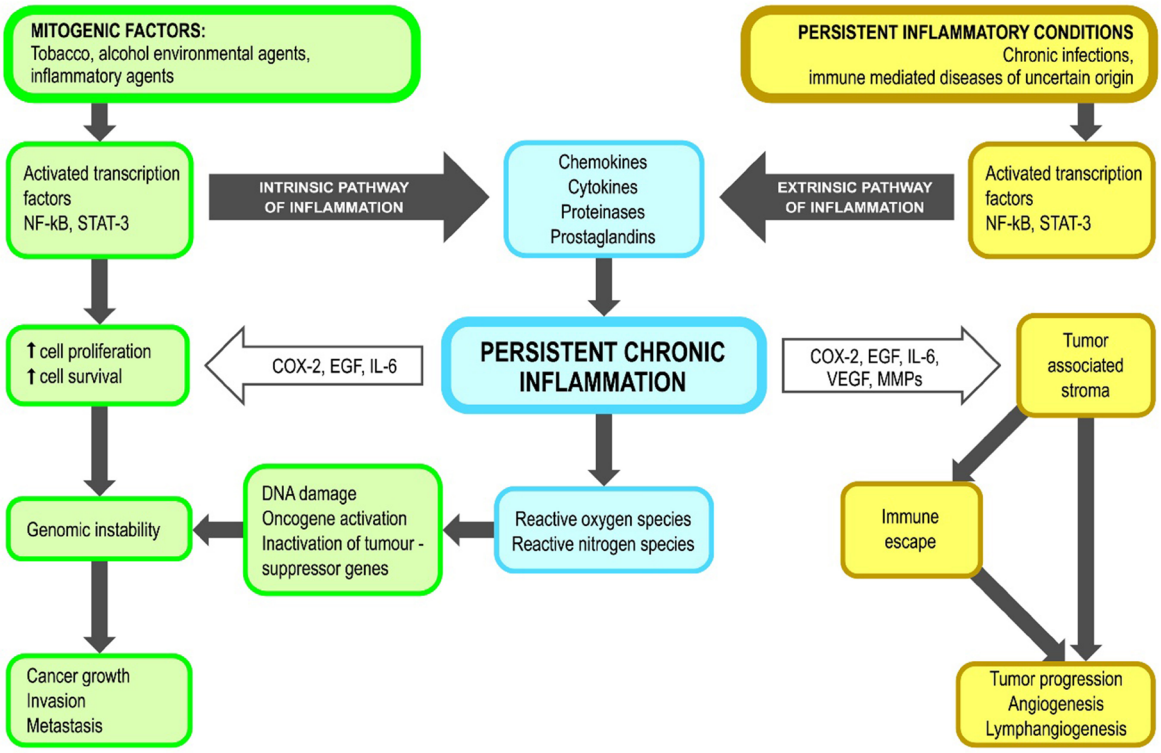

Figure 2 *Persistent chronic inflammation and carcinogenesis. Both intrinsic and extrinsic pathways of inflammation play an important role in cancer growth and progression via the production of various cytokines, growth factors and proteinases such as cyclooxygenase-2 (COX-2), epidermal growth factor (EGF), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) (adapted from Mantovani and Feller et al.).*[21, 22]

Previous studies have suggested that COX-2, in particular, plays a key role in carcinogenesis by inhibiting apoptosis, promoting tumor growth, invasion, and metastasis.[23,24] In addition, COX-2 has a crucial role in angiogenesis by regulating the expression of vascular endothelial growth factor (VEGF).[25] Overexpression of COX-2 has been found in many cancers such as head and neck, breast, gastric, colorectal, lung, and esophageal cancers.[16,17,22,26,27] Moreover, overexpression of COX-2 is associated with poor prognosis in colorectal and breast cancers.[16,28]

COX-2 and oral cancer

The results of previous investigations have strongly supported that overexpression of COX-2 plays an important role in OSCC carcinogenesis.[11,12,19,22,24,29] Intense COX-2 expression in cancer and surrounded inflammatory cells in OSCC is shown in Figure 3. Increased COX-2 expression is not only found in OSCC but also in oral dysplastic lesions in comparison with normal mucosa.[11] Moreover, there is a positive correlation between COX-2 expression and severity of dysplasia. Up-regulation of COX-2 also found in the potentially malignant disorders including oral lichen planus and oral submucous fibrosis.[29,30] These findings suggest that COX-2 plays roles in OSCC carcinogenesis and progression of premalignant lesions to malignancy. In addition, the expression of COX-2 in OSCC is associated with lymph node metastasis, tumor recurrences, poor prognosis and poor responses to radiotherapy.[12,22,31] It is known that *COX-2* gene polymorphisms affect the expression levels and enzymatic activity of COX-2, reflecting individual variations in inflammatory responses and susceptibility to cancers. COX-2 gene polymorphisms, particularly +837 T > C (rs5275) and -765G > C (rs20417), were shown to be associated with a risk of oral cancer.[32]

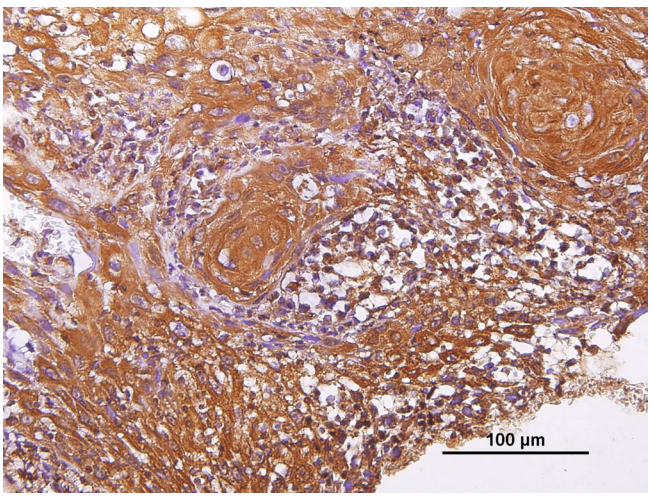

Figure 3 *The immunostaining of cyclooxygenase-2 (COX-2) in an oral squamous cell carcinoma (OSCC) case. Notably, the cancer and stromal inflammatory cells show an intense staining of COX-2 in the cytoplasm. These findings suggest a pivotal role of COX-2 produced by both cancer and stromal inflammatory cells in OSCC carcinogenesis (unpublished data).*

Targeting oral cancer with the COX-2 inhibitors

As aforementioned, numerous previous studies have revealed that OSCC uses the COX-2/PGE2 pathway to become more aggressive, progressive and further metastasized. Therefore, it is reasonable to inhibit the progression of OSCC by modulating the COX-2/PGE2 pathway.

Non-steroidal anti-inflammatory drugs (NSAIDs) particularly celecoxib, a drug that selectively inhibits COX-2, have drawn interests of many investigators to explore its potential in targeting many cancers including OSCC. In a successful colorectal cancer model, celecoxib can significantly reduce the risk of developing polyps in patients with familial adenomatous polyposis.[33] It is well known that cancer cells in general need to undergo EMT by downregulating some epithelial proteins such as E-cadherin and upregulating some mesenchymal proteins such as vimentin in order to become more mobilized and can further invade into the underlying or surrounding tissues.[15] By using the immunohistochemical method, the OSCC cells, which lost membranous E-cadherin staining, acquiring a cytoplasmic delocalization, demonstrated the overexpression of COX-2 suggesting a role of COX-2 in EMT of OSCC.[34] Interestingly, by targeting COX-2 in HNSCC, it was found that celecoxib has an anti-metastatic effect through suppression of EMT by restoring E-cadherin expression.[35] Celecoxib can also enhance chemosensitivity of oral cancer cells by blocking cell cycle progression.[36] In an oral premalignant model, celecoxib could improve the degree of dysplasia after 12 weeks of celecoxib therapy by employing COX-2, PGE2, and Ki67 as the biomarkers.[37] These findings suggest that celecoxib may also be used as the chemoprevention for oral cancer. Nevertheless, more studies are still needed to be explored in this area.

Recently, there was a suggestion that the combination of EGFR and COX-2 inhibitors may offer better outcomes for HNSCC treatment and prevention.[38] In an *in vitro* study, the combination of low concentrations of cetuximab, an anti-EGFR monoclonal antibody, and celecoxib significantly suppressed the proliferation, migration, invasion and reduced the production of PGE2 and VEGF in human OSCC.[39] In addition, this combined regimen could inhibit tumor growth in an OSCC xenograft nude mouse model. In a similar study model, the combined regimen of Sabutoclax, an inhibitor of all anti-apoptotic Bcl-2 proteins, and celecoxib synergistically inhibited the growth of OSCC *in vitro* and also significantly reduced OSCC tumor growth *in vivo*.[40]

In a clinical trial study, celecoxib showed a beneficial effect on the survival of patients with mobile tongue cancer.[41] When celecoxib was combined with accelerated radiotherapy in advanced head and neck cancer, the results revealed a significant decrease of the post-treatment serum VEGF level compared to the initial level especially in patients with high COX-2 expression in tumors.[42] Moreover, in the phase I study in recurrent or metastatic HNSCC, the patients appeared to be well tolerated with the combination of gefitinib, an EGFR inhibitor, and celecoxib suggesting further clinical

evaluation of the benefit of the use of EGFR and COX-2 inhibitors in HNSCC.[43] Similarly, a clinical trial on concurrent celecoxib, erlotinib, another EGFR inhibitor, and reirradiation demonstrated promising results in a population of patients with recurrent head and neck cancer who had a poor prognosis.[44]

Conclusion

Taken all evidence aforementioned, the author believes that targeting COX-2 by using COX-2 inhibitors is a potential and promising therapy for OSCC. A combination of celecoxib and cetuximab, gefitinib or erlotinib, in fact, may even provide synergistic anticancer effects. Since a long-term use of celecoxib may increase a risk of death by cardiovascular complications in patients[45], novel COX-2 inhibitors with minimized adverse effects need to be further explored. With this new paradigm shift in OSCC treatment, it is hopeful that the survival, morbidity and mortality rates in patients with OSCC will dramatically improve.

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