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REVIEW ARTICLE

Cyclooxygenase (COX)-2 as a potent molecular target for prevention and therapy of oral cancer

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Summary Cyclooxygenase (COX)-2 is one of two isoforms of COX that is the rate-limiting enzyme in the production of prostaglandin from arachidonic acid. It 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. Overexpression of COX-2 is known to inhibit apoptosis and immune surveillance, promote angiogenesis, increase cancer invasiveness and metastasis. Therefore, COX-2 is considered to be strongly involved in carcinogenesis and tumor growth. In fact, 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 through carcinoma *in situ*, with the elevation of cell proliferating activity. 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 or antisense RNA resulted in suppression of tumor cell growth and invasion *in vitro* and prevention of oral carcinogenesis by chemical carcinogens in animal models. In addition, combined use of selective COX-2 inhibitors was found to enhance synergistically the cytocidal 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.

Contents

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1. Introduction

Oral cancer is 1 of the 10 most frequent cancers in the world [1]. It accounts for approximately 2% of all cancers and 1% of all cancer deaths [2]. Squamous cell carcinoma (SCC) is the most common malignant tumor of the oral cavity, accounting for over 90% of the malignant neoplasms in this region [3], and is thought to arise from a progressive dysplasia of the oral mucosa. The incidence of regional lymph node metastasis in oral SCC is comparatively high. The outcome of the patients with lymph node metastasis is poor, with overall 5-year survival rate of approximately 25% [4]. Despite of recent advances in diagnosis and the surgical, radiotherapeutic, and chemotherapeutic treatments of oral cancer, 5-year survival rate has improved only marginally [3]. Furthermore, recent epidemiologic data indicated that the incidence of oral cancer is increasing [2,5]. Therefore, the setting of new molecular targets is necessary for diagnosis, prevention and treatment of oral cancer.

In recent years, overexpression of cyclooxygenase (COX)-2 has been reported in a variety of cancers, including those arising in the colon [6—8], stomach [9], breast [10], lung [11], esophagus [12], pancreas [13], urinary bladder [14], prostate [15] and head and neck [16—18]. COX is the rate-limiting enzyme in the production of prostaglandin from arachidonic acid [19]. Two isoforms of the COX enzyme exist, COX-1 and COX-2, and they have been postulated to be target molecules for non-steroidal anti-inflammatory drugs (NSAIDs). COX-1 is constitutively expressed in most tissues and appears to be a housekeeping enzyme responsible for various physiological functions, such as cytoprotection in the stomach, vasodilation in the kidney, and production of pro-aggregatory prostanoid thromboxane by platelet. On the other hand, COX-2 is induced by stimuli such as mitogens, cytokines, growth factors and tumor promoters, and has been elucidated to be involved in cancer development and pathogenesis such as apoptosis [20], immune surveillance [21], angiogenesis [22], invasion and metastasis [23], and cell differentiation (Fig. 1) [24]. In this review, the relations among expression of COX-2 and carcinogenesis of the head and neck region including oral cavity, malignant phenotype and prognosis of the patients are focused. In addition, the possibility of COX-2 as a potent molecular target for prevention and therapy of oral cancer is discussed.

2. COX-2 expression in precancerous and cancerous lesions of the head and neck including oral cavity

Initial observations for overexpression of COX-2 in precancerous and cancerous lesions were done in colorectal adenomas and carcinomas [6—8]. This was based on the epidemiological studies that the relative risk for colorectal cancer was reduced by a 40-50% in persons who regularly use aspirin and other NSAIDs [25—27]. Since then, overexpression of COX-2 has been demonstrated in various cancer tissues, mainly in adenocarcinomas. In these studies, COX-2 protein was primarily distributed from the cytoplasmic perinuclear region to the cytoplasmic matrix of tumor cells. With respect to head and neck cancer, Chan et al. [16] have first reported the up-regulation of COX-2 in head and neck squamous cell carcinoma (HNSCC). They found by quantitative reverse transcription-PCR that mean levels of COX-2 mRNA were increased by nearly 150-fold in HNSCC ($n = 24$) compared with normal oral mucosa ($n = 17$), and normal-appearing epithelium adjacent to SCC ($n = 10$) showed about a 50-fold increase of COX-2 mRNA compared with normal oral mucosa. Immunoblotting and immunohistochemical analyses gave the supportive results. We have also demonstrated increased expression of COX-2 in human salivary gland tumors including adenomas (SGA) and carcinomas (SGC) [17]. COX-2 protein was detected in 27 of 30 SGA (90%), except for three myoepitheliomas, and in all cases of SGC (100%) at various intensities and in various fashions. Thirteen SGA (43%) and 36 SGC (90%) showed strong COX-2 immunoreactivities. These find-

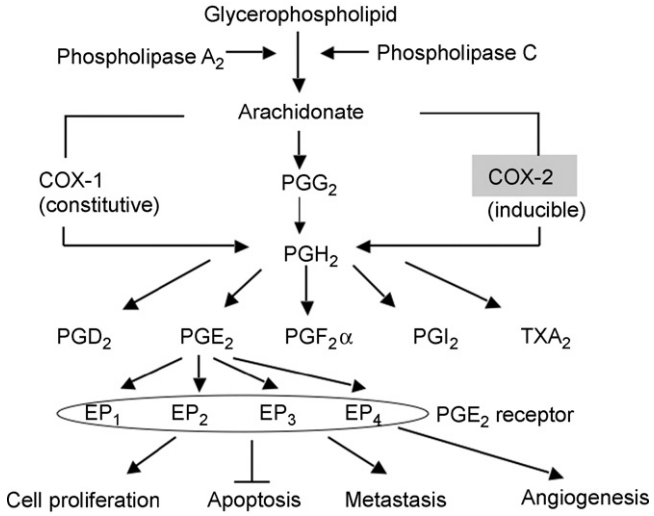

Figure 1 Pathway of prostanoid synthesis from arachidonic acid, and the involvement of COX-2 in cancer development. Arachidonic acid is released from membrane phospholipid by phospholipase A_2. COX-1 or COX-2 converts arachidonic acid to prostaglandin (PG) G_2 followed by conversion to PGH_2 into other PG isoforms or thromboxane (TX) A_2. Among them, PGE_2 is the major prostanoid and exhibits a variety of biologic activities via its EP receptors.

ings were consistent with those reported for colorectal tumors by Eberhart et al. [6]. When compared COX-2 expression between SGC and HNSCC, the intensity of COX-2 expression was clearly lower in HNSCC than in SGC. Hida et al. [11] reported a similar result that more than two-thirds of adenocarcinomas in the lung showed overexpression of COX-2 whereas SCCs of the lung were detected infrequent and low expression. They speculated that the increased COX-2 expression might be a characteristic of human cancers derived from glandular epithelium.

Head and neck cancer including oral cancer is considered to develop by accumulated genetic alterations [28—30], and the major pathway is cancerization from precancerous lesions such as intraepithelial dysplasia in oral leukoplakia and erythroplakia. Shibata et al. [31] examined COX-1 and -2 expression by Western blot and immunohistochemical analyses for 65 dysplasias and 50 SCCs, and reported that the labeling indices for COX-1 and -2 were higher in dysplasias than in SCCs and those of COX-2 but not COX-1 correlated with the histological grade of dysplasias, being highest in the severe dysplasias. Also, they stated that COX-2 expression was significantly inversely correlated with the histological differentiation of the SCCs. From these results, they concluded that the expression of COX-1 and -2 is correlated with early stage tumorigenesis and cellular differentiation of SCCs in oral dysplasia-carcinoma sequence. Recently, we have examined the expressions of COX-2 and DNA topoisomerase (DNA-Topo) IIα as an index of cell proliferating activity immunohistochemically for 60 intraepithelial dysplasias (IEDs), 12 carcinomas *in situ* (CISs), 72 SCCs, 10 undifferentiated carcinomas (UCs), and 10 epithelial hyperplasias (EHPs) in the oral mucosa and found that 41% of IEDs, 67% of CISs, 74% of SCCs and 86% of UCs demonstrated increased COX-2 expression with elevated DNA-Topo IIα labeling index (Figs. 2 and 3) [32]. Increased COX-2 expression correlated with elevated DNA-Topo IIα labeling index, indicating that COX-2 may contribute to malignant transformation and tumor growth. A similar high COX-2 expression was observed in EHPs, but DNA-Topo IIα labeling index was very low. In contrast with Shibata's paper, we observed no significant difference in expression of COX-2 among mild, moderate and severe dysplasia, and COX-2 expression was increased as tumor differentiation was decreased. Similar results that there is no significant difference of COX-2 expression in the degrees of intraepithelial dysplasia were reported by Renkonen et al. [33] and Sudbo et al. [34] In particular, Sudbo et al. stated that COX-2 expression was correlated to DNA content (DNA aneuploidy) as a genetic risk marker of oral cancer. With respect to COX-1 expression in HNSCC, we did not find the overexpression in cancer tissues. COX-1 protein was diffusely stained in both dysplastic and carcinoma cells, and mainly observed in lymphocytes, macrophages, vascular endothelial cells and striated muscles [32]. Therefore, it is needed to accumulate more evidences for COX-1 expression in oral precancerous and cancerous lesions.

The relationship between COX-2 expression and tumor differentiation in SCCs is still controversial. In the lung [11], esophagus [35] and larynx [36], 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 [33] and of the esophagus [37] that more undifferentiated carcinoma (histological grade III) had significantly stronger COX-2 expression than grade I or II cases. In our recent study, we have reported

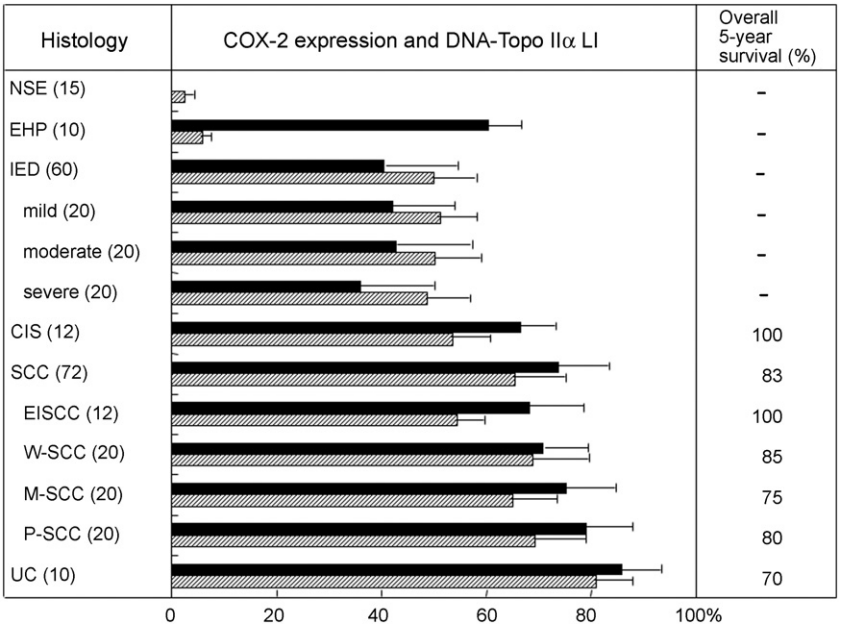

Figure 2 COX-2 expression and DNA-Topo IIα labeling index in oral precancerous and cancerous lesions and outcome of the patients. The percentage of COX-2-positive (black bar) and DNA-Topo IIα-positive cells (hatched bar) were determined immunohistochemically by counting a total 2000 cells in 10 randomly selected fields of each section examined at 400× magnification. NSE, normal squamous epithelium; EHP, epithelial hyperplasia; IED, intraepithelial hyperplasia; CIS, carcinoma *in situ*; SCC, sqamous cell carcinoma; EISCC, early invasive squamous cell carcinoma; W-SCC, well-differentiated squamous cell carcinoma; M-SCC, moderately differentiated squamous cell carcinoma; P-SCC, poorly differentiated squamous cell carcinoma; UC, undifferentiated carcinoma (cited from Ref. [32]).

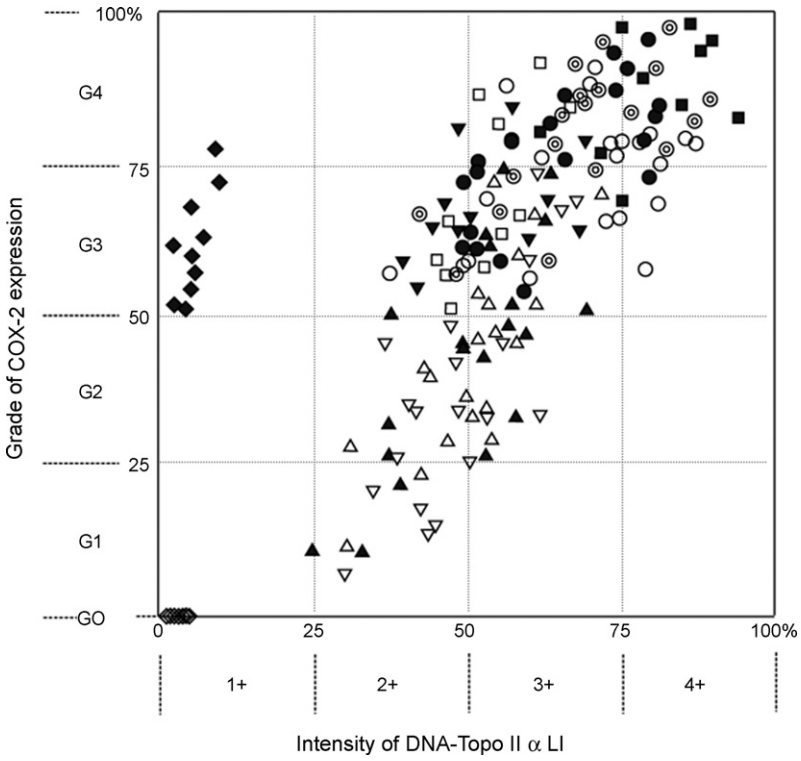

Figure 3 Relationship between expression of COX-2 protein and DNA-Topo IIα labeling index in oral precancerous, cancerous and hyperplastic lesions. (◇) NSE; (◆) EHP; (△) mild IED; (▲) moderate IED; (▽) severe IED; (▼) CIS; (□) EISCC; (○) W-SCC; (●) M-SCC; (◎) P-SCC; (■) UC. Statistical significance was $r = 0.74$, $p < 0.001$ in χ^2 test (cited from Ref. [32]).

that treatment of human oral SCC25 cells with sodium butyrate, a differentiation-inducing agent, caused promotion of cell differentiation and suppression of cell growth and COX-2 expression [38]. These findings supported our histological data in tumor differentiation and COX-2 expression.

3. COX-2 expression and prognostic significance in oral cancer

Accumulating evidences indicate that expression of COX-2 is closely related to overall survival, along with prognostic variables such as tumor size, tumor differentiation, lymph node metastasis, and disease stage [39—44]. However, several studies have reported that overexpression of COX-2 does not significantly correlate with survival [36,45—48]. Whether overexpression of COX-2 is associated with poor prognosis thus remains controversial. Only 3 out of these previously published studies were performed in patients with HNSCC. Two of these studies, one in HNSCC by Gallo et al. [43] and the other in oropharyngeal SCC by Chang et al. [44], reported that COX-2 overexpression is related to poor survival, whereas the other in laryngeal SCC by Ranelletti et al. [36] found that the level of COX-2 negatively correlated with both overall survival and relapse-free survival. Gallo et al. [43] described in their study using 52 patients with HNSCC that the most significant prognostic factors were presence of lymph node metastasis, tumor vascularization associated with expression of vascular endothelial growth factor (VEGF), COX-2 expression and PGE_2 levels. Chang et al. [44] reported by the retrospective cohort study using 82 patients with SCC that COX-2 was the most important predictor of poor survival in multivariate analysis. In contrast, Ranelletti et al. [36] described in their report studying 61 patients with laryngeal SCC that the significant prognostic factors for relapse-free and overall survivals were high histopathological grading, lymph node involvement, low levels of COX-2 expression, and high levels of EGFR expression. In our recent study [49], we compared the expressions of COX-2 and DNA-Topo IIα immunohistochemically between 80 patients who had oral carcinoma with lymph node metastasis and 80 patients who had oral carcinoma without lymph node metastasis. These groups were matched with respect to sex, age, tumor site, T category, tumor growth pattern, and tumor differentiation. As a result, COX-2 expression in primary lesions was higher in cases with lymph node metastasis than in those without lymph node metastasis. An increase in tumor size was associated with increased COX-2 expression. In most cases with lymph node metastasis, COX-2 expression was higher in metastatic lesions than in primary lesions. As COX-2 expression increased, the DNA-Topo IIα labeling index significantly increased and the overall 5-year survival rate decreased (Table 1, Fig. 4). Our results supported the hypothesis that overexpression of COX-2 is associated with poor survival and closely related to lymph node metastasis.

4. COX-2 expression and malignant phenotypes *in vitro*

Overexpression of COX-2 causes excess production of prostaglandins, and induces an increase of cell proliferation and

Table 1 The percentage of COX-2-positive and DNA-Topo IIα-positive tumor cells in primary and metastatic lesions of oral carcinoma

	COX-2 expession (%)		DNA-Topo IIα LI (%)	
Normal muicosa (n = 20)	0		6.79 ± 4.23	
Primary ;esopms				
without lymph node metastasis (n = 80)	28.4 ± 27.6	p = 0.039	35.2 ± 12.1	p = 0.017
without lymph node metastasis (n = 80)	37.3 ± 38.1	p = 0.026	59.5 ± 13.0	p = 0.031
Metastatic lessions	52.5 ± 39.2		80.0 ± 13.5	

Note: Statistical analysis was done by using the Fisher's exact test (cited from Ref. [49]).

decrease of apoptosis, mostly mediated by PGE_2 and its receptor $EP_{1\text{-}4}$ (Fig. 1). This overexpression has been elucidated to contribute to a variety of malignant phenotypes and cancer pathogenesis. Tsujii et al. [20] reported that rat intestinal epithelial cells transfected with a COX-2 expression vector were resistant to butyrate-induced apoptosis, had elevated BCL2 protein expression, and increased adhesion to extracellular matrix. These phenotypic changes were reversed by sulindac sulfide (a COX inhibitor). They also reported that human colon cancer cells (Caco-2) overexpressing COX-2 produce prostaglandins, proangiogenic factors, and stimulate both endothelial migration and tube formation. This effect was inhibited by antibodies to anti-angiogenic factors, by NS-398 (a selective COX-2 inhibitor), and by aspirin [22]. In addition, these cells acquired increased invasiveness and metastatic potential compared with the parental Caco-2 cells or the vector-transfected control cells, via activation of metalloproteinase (MMP)-2 and membrane-type metalloproteinase (MT-MMP). Increased invasiveness was reversed by treatment with sulindac sulfide [23]. Huang et al. [21] reported using A549 non-small cell lung cancer cells that tumor-derived PGE_2 modifies cytokine balance and inhibits host immunity. Excess of PGE_2 production by increased expression of COX-2 in A549 cells caused up-regulation of interleukin (IL)-10 in lymphocytes and macrophages and down-regulation of IL-12 production in macrophages via stimulation of IL-1β. Leong et al. [24] reported that in human epidermis as well as in human keratinocyte cultures, the COX-2 expression induces normal keratinocyte differentiation.

In head and neck cancer, the significance of COX-2 expression in relation to tumor cell migration, invasion and metastasis is not fully understood. A few *in vitro* studies have been reported about COX-2 expression and cell proliferation

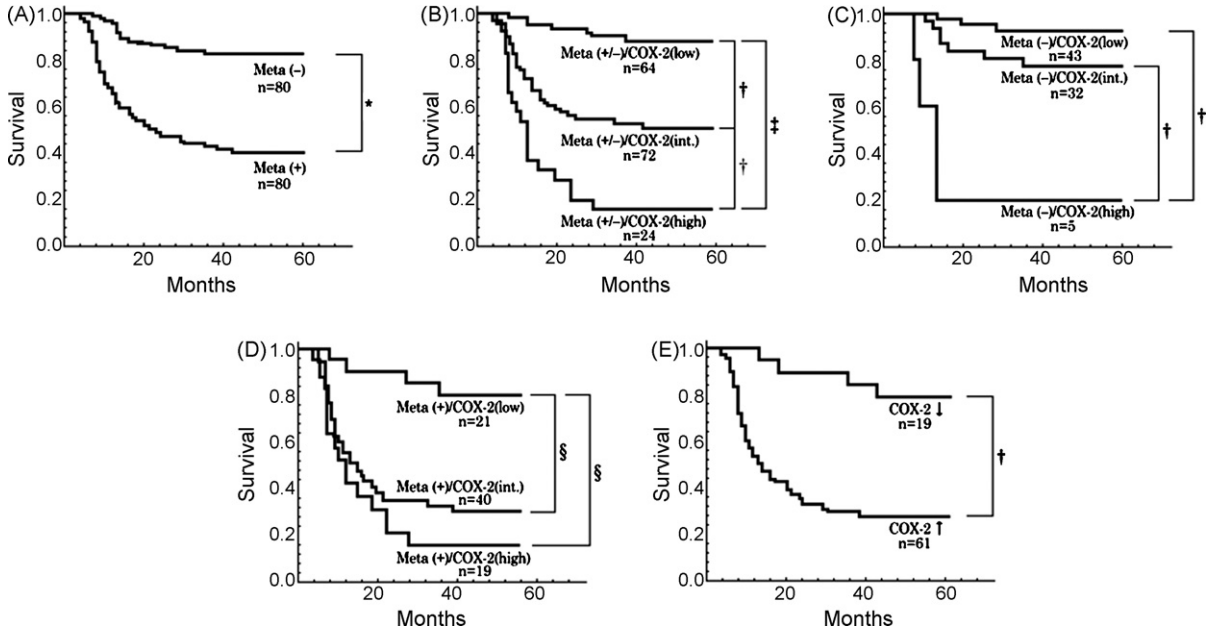

Figure 4 Overall 5-year survival of patients who had oral carcinoma with or without lymph node metastasis or with various intensities of COX-2 expression. (A) Survival of patients with or without lymph node metastasis ($n = 160$). (B) Survival of patients with various intensities of COX-2 expression in primary lesions ($n = 160$). (C) Survival of patients without lymph node metastasis according to COX-2 expression in primary lesions ($n = 80$). (D) Survival of patients with lymph node metastasis according to COX-2 expression in primary lesions ($n = 80$). (E) Survival of patients whose COX-2 expression in metastatic lesions was increased or decreased as compared with that in primary lesions ($n = 80$). $^{*}p < 0.01$, $^{\dagger}p < 0.005$, $^{\ddagger}p < 0.001$, $^{\S}p < 0.0005$ (cited from Ref. [49]).

[50,51] and invasiveness of tumor cells [52] in HNSCC. Sumitani et al. [50] reported that proliferation of oral SCC cell lines NA and HSC-4 constitutively expressing COX-2 was suppressed by NS-398 or COX-2 antisense oligonucleotide, and Toyoshima et al. [51] reported that these inhibitors induced G0/G1 cell arrest in NA cells via cyclin-dependent kinase inhibitor p21. Kinugasa et al. [52] also reported that NS-398 and COX-2 antisense oligonucleotide suppressed the invasiveness of NA and HSC-4 cell lines in a Matrigel invasion assay via down-regulation of MMP-2, MT1-MMP, and CD44. We reported that cell growth of human tongue carcinoma cell line SCC25 producing ample amount of PGE_2 was inhibited by celecoxib, a selective COX-2 inhibitor, and sulindac, but exogenous addition of PGE_2 resulted in an increased cell growth of SCC25 even under the celecoxib-treated condition [53]. Recently, we have reported that human KB carcinoma cells transfected with COX-2 cDNA (KB/COX-2) showed a similar growth rate *in vitro* compared to mock-transfected control cells (KB/neo), but demonstrated significantly increased ability of cell migration and invasion in wound healing assay and Matrigel invasion assay, via up-regulation of MMP-9, MMP-2 and MT1-MMP and down-regulation of tissue inhibitor of metalloproteinases (TIMP)-1 and TIMP-2 [54]. Reorganization of the actin cytoskeleton of the cell is the primary mechanism of cell motility and is essential for most types of cell migration. The actin reorganization is regulated by Rho family small GTPases as Rho, Rac and Cdc42 [55]. Therefore, KB/COX-2 cells were examined for the Rho family small GTPases by Western blot analysis. Consequently, KB/COX-2 cells were found to show the elevated expression of RhoA and Rac1 but not of Cdc42 as compared with KB/neo cells [54].

5. COX-2 expression in oral carcinogenesis in animal models and chemoprevention by selective COX-2 inhibitors

Numerous experimental rodent models as well as epidemiological studies have shown an inverse relationship between NSAIDs intake and colon cancer development [25,56—58]. Clinical trials with NSAIDs in patients with familial adenomatous polyposis (FAP) have clearly demonstrated that NSAID treatment caused regression of preexisting adenomas [59]. A variety of studies for colon cancer in genetic animal models including mutant mice with deletion of tumor suppresser gene APC (adenomatous polyposis coli) [60] and carcinogen-treated animal models have also indicated a significant reduction in tumor multiplicity by NSAID treatment [61]. Several animal studies concerning COX-2 expression in carcinogen-induced oral carcinogenesis and chemoprevention with selective COX-2 inhibitors have been reported [62—65]. Shiotani et al. [62] and Yamamoto et al. [63] reported that 4-nitroquinoline-1-oxide (4-NQO)-induced squamous cell dysplasias and carcinomas of the tongue in Fisher 344 rats demonstrated increased COX-2 expression, and the feeding of a selective COX-2 inhibitor, nimesulide or etodolac, to rats decreased the incidence and multiplicity of tongue lesions in a dose-dependent manner. Yoshida et al. [64] also reported that nimesulide exerts chemopreventive ability against 4-NQO-induced tongue tumorigenesis through inhibition of cell proliferation activity in conjunction with modification of COX-2 and iNOS expression of the target lesions. We have recently reported that COX-2 expression was increased during hamster cheek pouch carcinogenesis with 9,10-dimethyl-1,2-benzanthracene (DMBA), and the feeding of celecoxib, a selective COX-2 inhibitor, delayed the onset of tumor formation and tumor growth, resulting in a prolonged survival of animals. These effects were considered to be at least due to increased induction of apoptosis in the tumor parenchyma and significantly reduced angiogenesis in the stroma [65]. Similar data were shown by Nishimura et al. [66]. They examined the anti-tumor effect of a selective COX-2 inhibitor, JTE-522, in nude mice xenografted with human KB carcinoma cells and indicated that the effect was caused by anti-angiogenesis action, cell cycle arrest and inhibition of telomerase activity of the tumor cells, resulting in an induction of apoptosis.

6. Possibility of clinical use of COX-2 inhibitors in treatment of oral cancer

In recent years, several preclinical studies for application of selective COX-2 inhibitors in cancer treatment have been performed concomitant with development of more selective COX-2 inhibitors. As described before, single administration of COX-2 inhibitors may be feasible to benefit the prevention of colon carcinogenesis, especially for FAP patients, but it is unlikely in application to precancerous lesions such as leukoplakia and erythroplakia for the prevention of oral carcinogenesis. In the clinical setting for head and neck cancer including oral cancer, combination therapy of COX-2 inhibitors with irradiation, anti-cancer drugs or inhibitors of epidermal growth factor receptor (EGFR) may be possibly applicable [67]. Amirghahari et al. [68] reported that NS-398 demonstrated the radiosensitising effect on human HNSCC cell line HEp3 via inhibition of radiation-induced up-regulation of COX-2 protein expression. Raju et al. [69] 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. As another mechanism, Gorski et al. [70] proposed that the anti-angiogenic actions of COX-2 inhibitors produced rediosensitisation via blockade of the VEGF stress response. Also, Milas et al. [71] described that NSAIDs and selective COX-2 inhibitors increased tumor radioresponse by restoring immunoreactivity. 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 [72].

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 al. [73] reported that nimesulide induced apoptosis and enhanced cytotoxicity of cisplatin and etoposide (VP-16) in lung cancer cells. Duffy et al. [74] reported that COX-2 inhibitors showed a concentration-dependent synergistic anti-tumor effect on human lung cancer cell lines and a human leukemia line in combination with anthracycline

(doxorubicin, daunorubicin and epirubicin), teniposide, etoposide and vincristine. We also 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 [53]. In *in vivo* studies, Hida et al. [75] reported that combined use of JTE-522 and conventional anti-cancer agents such as docetaxel and vinorelbine significantly increased the efficacy of both *in vitro* and *in vivo* growth inhibition in human lung cancer cells. Nakata et al. [76] demonstrated that celecoxib enhanced the anti-tumor efficacy of docetaxel 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, Altorski et al. [77] found that the addition of celecoxib enhanced the response to preoperative paclitaxel and carboplatin. Therefore, combination of COX-2 inhibitors with chemotherapeutic agents and/or irradiation is considered to have a high potential for oral cancer treatment.

Activation of epidermal growth factor receptor (EGFR) has been linked to cancer. Expression of EGFR has been documented to be deregulated in oral mucosa during head and neck tumorigenesis and up-regulated in HNSCC [78]. Its overexpression has been known to correlate with poor prognosis in head and neck cancer [79]. Thus, EGFR is also a therapeutic target for treatment of head and neck cancer. Chen et al. [80] reported that combination of EGFR-selective tyrosine kinase inhibitors AG1478 or ZD1839 (Iressa or gefitinib) with celecoxib either additively or synergistically inhibited growth of five HNSCC cell lines by inducing G1 arrest and apoptosis and suppressing capillary formation of endothelium. They also reported that combination use of ZD1839 and celecoxib significantly inhibited tumor growth in nude mice xenografted with HNSCC cell line Tu212 [81]. Since Milas et al. [82] found that anti-EGFR antibody (C225 or cetuximab) enhanced *in vivo* radioresponse in nude mice xenografted with human A431 carcinoma cells, combination therapy of inhibitors of EGFR tyrosine kinase and COX-2 with radiation and/or chemotherapeutic agents may become useful strategies for treatment of oral cancer. In fact, it has been reported as a chemoprevention trial that a phase III randomized placebo-controlled study using COX-2 inhibitor celecoxib and EGFR inhibitor EKB-569 in patients with aneuploid dysplastic oral leukoplakia is in advanced planning stages [83].

Selective COX-2 inhibitors are advantageous not only in their high ability to enhance tumor response to radiotherapy and chemotherapy but also in their lower association with gastrointestinal complications, as compared with standard NSAIDs including COX-1 inhibitors [84]. However, it has recently been demonstrated that selective COX-2 inhibitors (e.g. rofecoxib, celecoxib) may be prothrombotic and have a higher risk of myocardial infarction than COX-1 inhibitors (e.g. naplofen) [85]. Although several investigators have shown no significant difference between them [86], the toxicity has been a concern in future clinical study. At least, celecoxib has already been permitted in clinical use in the United States, and also permitted in Japan in June 2007.

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