Minireview

Interaction between epidermal growth factor receptor– and cyclooxygenase 2–mediated pathways and its implications for the chemoprevention of head and neck cancer

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Abstract

Head and neck squamous cell carcinoma is a well-known model for chemoprevention studies because of its field cancerization effect, its multistep carcinogenesis process, and the easy accessibility of biopsies to target lesions. With new understandings of head and neck carcinogenesis and the development of molecular targeted therapy, chemoprevention trials for head and neck squamous cell carcinoma have been rapidly updated. Cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors are gaining significant attention as potential chemopreventive agents. Both COX-2 and EGFR are involved in head and neck carcinogenesis. Targeting COX-2 and EGFR separately has shown promising antitumor activity. Recently, combinations of COX-2 and EGFR tyrosine kinase inhibitors have been reported to show synergistic/additive effects in preclinical studies. Because COX-2 and EGFR tyrosine kinase inhibitors are toxic as single agents in clinical trials, the combination of COX-2 and EGFR tyrosine kinase inhibitors used at lower doses seems more promising than monotherapy with either as a novel strategy in head and neck cancer chemoprevention. [Mol Cancer Ther 2005;4(9):1448–55]

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Introduction

Approximately 40,000 cases of new head and neck cancer (HNC) develop annually, accounting for ~3% of all new cancers in the United States (1), and 90% of these are squamous cell carcinoma [head and neck squamous cell carcinoma (HNSCC)]. Due to its location and anatomic complexity, HNC causes almost inevitable functional and social impairment even before it becomes life threatening. Despite great advances in therapy, the overall survival rate for patients with HNC has not improved significantly, emphasizing the importance of preventive intervention (2–4).

Chemoprevention can be defined as the use of specific agents to suppress, reverse, or prevent carcinogenesis, thus stopping the progression to invasive cancer by modulating the carcinogenic process or by removal (apoptosis) of premalignant cells (5, 6). Recently, the concept of chemoprevention has been substantially incorporated into cancer treatment goals. Progress in clinical trials has shown that the combined preventive approach is more effective than single-agent chemoprevention.

This review will briefly summarize chemopreventive approaches in HNSCC before highlighting a novel and promising chemopreventive modality combining cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors.

Chemoprevention for HNC

HNSCC is an excellent model for the chemopreventive approach in several aspects. First, HNSCC is notorious for its high tendency to develop second primary tumors, one of the main reasons for the typical dismal outcome of this cancer. The lifetime cumulative risk of second primary tumor is >20% and has been reported in up to 47% in patients with previously treated laryngeal cancer (2, 7). This multicentricity is best explained by the fact that the whole mucosa of the upper aerodigestive tract is affected by the same carcinogens, most likely tobacco and alcohol (field cancerization; ref. 8). Second, HNSCC well exemplifies multistep carcinogenesis with stepwise accumulations of genetic alterations (9, 10). Pathologically, the course follows from normal epithelium, hyperplasia, and dysplasia to invasive HNSCC. Multicentricity and multistep carcinogenesis provides the rational background for a chemopreventive

approach. In addition, HNC exhibits clinically well defined premalignant lesions, such as oral leukoplakia and erythroplakia, to be targeted. These can be easily identified compared with premalignant lesions in other organ sites and provide accessibility for macroscopic and microscopic evaluation or biomarker evaluation of lesions.

HNSCC has been one of the main models for chemopreventive approaches and important issues have been learned from chemopreventive clinical trials. The most extensively studied chemopreventive agents in HNC areretinoids (11). The first randomized clinical trial was conducted by Hong et al. (11) using a high dose of 13-*cis*-retinoic acid (13-cRA) for a short period in patients with oral premalignant lesions, which showed significant clinical and pathologic responses. In spite of the encouraging results, this trial raised two important issues common in chemopreventive clinical trials. Dose-related toxicity was an important obstacle to treating patients on a long-term basis, and the remission induced by short-term treatment did not last long after cessation of treatment. This observation led to a subsequent low-dose maintenance trial. Lippman et al. (5) treated patients with oral premalignant lesions with high-dose 13-cRA for 3 months and switched to either low-dose 13-cRA or β-carotene for maintenance. Although the results showed that using low-dose 13-cRA for maintenance could effectively repress disease progression, the long-term follow-up failed to show a difference in the cancer development rate in both groups (11). In terms of prevention of second primary tumor, the first phase III clinical trial using 13-cRA showed a statistically significant suppression of second primary tumor, although there was no significant difference in survival. Subsequent randomized clinical trials using retinoids have failed to show a difference in the development of second primary tumor or survival (11).

Obtaining long-lasting efficacy with low toxicity has been an important issue, provoking combinations of retinoids with other agents or searches for new chemopreventive agents. Other chemopreventive agents that have shown preventive effects include selenium and vitamin E. Recently, Papadimitrakopoulou et al. (12) reported that a combination of 13-cRA, vitamin E, and IFN-α restored advanced laryngeal premalignant lesions. Shin et al. (13, 14) showed that the same combination suppressed the development of second primary tumor and/or recurrence, and achieved an excellent survival rate in stage III/IV HNSCC, making this a promising combination chemopreventive approach. With a better understanding of relevant molecular changes in each carcinogenic step of HNC and increased availability of specific molecular targeting agents, chemoprevention in HNC has evolved to a new era. New agents in this context include COX inhibitors, EGFR tyrosine kinase inhibitors, farnesyl transferase inhibitors, and others (11).

Rationale for Blocking COX-2 and EGFR Pathways in Chemoprevention of HNC

Selection of chemopreventive agents requires consideration of several criteria. Biological efficacy impeding the carcinogenic process, selectivity for transformed tissue, minimal toxicity, and the ability to obtain good patient compliance are essential. As new potential agents, COX-2 inhibitor and EGFR inhibitor show promise for chemoprevention of HNC. Both the COX-2 and EGFR signaling pathways play major roles in head and neck carcinogenesis (15, 16). Both COX-2 and EGFR are overexpressed in premalignant and malignant tissues of the head and neck compared with normal tissue (17–20). Blocking these pathways has already shown promising antitumor activity. In addition, both the COX-2 and EGFR inhibitors used in clinics are orally bioavailable and their side effects are well tolerated at clinically relevant dosages (21–23).

COX-2 Pathway in Carcinogenesis

COX-1 and COX-2 catalyze prostanoid synthesis from arachidonic acid. COX-1 is constitutively expressed in nearly all normal tissues and has a beneficial housekeeping role. COX-2 is undetectable (or at low levels) in most normal tissues but is rapidly induced in response to inflammatory or mitogenic stimuli, including cytokines, growth factors, and tumor promoters (24, 25).

COX-2 has been implicated in carcinogenesis ever since the discovery that intake of nonsteroidal anti-inflammatory drugs that inhibit COX activity also decrease the relative risk for development of colorectal cancer (26). There is also direct evidence for the carcinogenic role of COX-2. Oshima et al. (27) showed that selective genetic elimination of COX-2 protected *APC* tumor suppressor gene–deleted mice from developing intestinal tumors. In humans, COX-2 overexpression has been found in premalignant and malignant lesions in several tumors (17, 18, 25; for review, see 28). In HNC, there is a stepwise increase in COX-2 expression through the normal, hyperplastic, dysplastic, and invasive carcinoma stages, suggesting its carcinogenic role in HNC (17, 18, 29).

COX-2 overexpression contributes to many aspects of carcinogenesis, such as inhibition of apoptosis, promotion of cell proliferation, induction of angiogenesis, and increasing invasiveness, mainly through increasing the amount of prostaglandins, including prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, PGD_2, TXA_2, PGI_2, and PGJ_2 (28, 30). Prostaglandins exert their effect mainly by binding to G-protein–coupled receptors, including EP-1, EP-2, EP-3, and EP-4, on cell surfaces (for review, see ref. 31). Each is specifically recognized by certain prostaglandins and activates signaling transduction from extracellular signal regulated kinase (32), phosphatidyl inositol 3-kinase/AKT, and cyclic AMP/protein kinase A (32–35). Alternatively, cyclopentenone prostaglandins, such as PGJ_2, can bind to the nuclear transcription factor peroxisome proliferator-activated receptor, which regulates expression of several genes involved in cell proliferation (36).

EGFR Signaling Pathway in Cancer Progression

EGFR, a surface receptor with intrinsic tyrosine kinase activity, is one of several known pivotal intermediates in many epithelial malignancies (37). It belongs to the erbB growth factor receptor family. The ligands binding to the

extracellular domain of EGFR induce homodimerization of EGFR or heterodimerization with other members of the erbB growth factor receptor family, activating the intrinsic tyrosine kinase and its downstream signaling molecules (38). Potential EGFR downstream signaling pathways include Ras/mitogen-activated protein kinase, phosphatidyl inositol 3-kinase, phospholipase Cγ, the Src kinase family, Janus kinase, signal transducers, activators of transcription, and others (39). Activation of EGFR is involved in pertinent pleiotropic cellular processes, such as cell proliferation, apoptosis, differentiation, angiogenesis, and motility (37–43).

Overexpression of EGFR has been frequently reported in human malignant neoplasms (44). In HNC, expression of EGFR and its ligands, transforming growth factor-α or epidermal growth factor, are up-regulated in histologically normal epithelium adjacent to invasive cancer compared with control normal epithelium in individuals without cancer. Dysplastic lesions overexpress EGFR, and invasive cancer displays a more jumped-up pattern of overexpression (19, 20). All this implicates the EGFR signaling pathway in the early stages of head and neck carcinogenesis and progression. EGFR overexpression is also associated with a poorer prognosis in HNC patients (45).

Interaction between EGFR and COX-2 Pathways

Whereas the pathways by which EGFR and COX-2 contribute to carcinogenesis have been separately considered and targeted, increasing evidence indicates a tight connection between these two pathways (Fig. 1).

EGFR and COX-2 signaling pathways form a positive feedback loop. Activation of EGFR has been shown to induce increased COX-2 expression in various normal and tumor cell lines, including HNSCC cell lines (46–54). Both transforming growth factor-α and epidermal growth factor, ligands of EGFR, were found to induce COX-2 expression (46–50). Expression of COX-2 is regulated at both the transcriptional and posttranscriptional level. The signaling pathway involved in COX-2 induction via EGFR varies depending on the type of cells and inducers, but the ras/raf/mitogen-activated protein kinase signaling pathways mainly contribute to both increased transcriptional and posttranscriptional control. One explanation for the linkage between EGFR/mitogen-activated protein kinase and transcriptional activation of COX-2 may be the activation of transcription factors such as cyclic AMP response element-binding protein/activating transcription factor and activator protein-1 by mitogen-activated protein kinase signaling (25, 46, 51, 54). Binding sites for these transcription activators have been identified in the COX-2 promoter region.

On the other hand, COX-2 induces transactivation or increased expression of EGFR (55, 56). Transactivation of EGFR by PGE_2, a major prostaglandin involved in carcinogenesis, has been well documented but the process seems to be quite complex and cell type dependent. Pai et al. (56) showed that PGE_2 transactivated EGFR and triggered the activation of extracellular signal regulated kinase-2 pathways in normal gastric epithelial cells and colon cancer cells, inducing cell proliferation *in vitro* and *in vivo*. The G-protein coupled receptor, to which the major prostanoids receptors belong, is involved in EGFR transactivation. The mechanism by which G-protein coupled

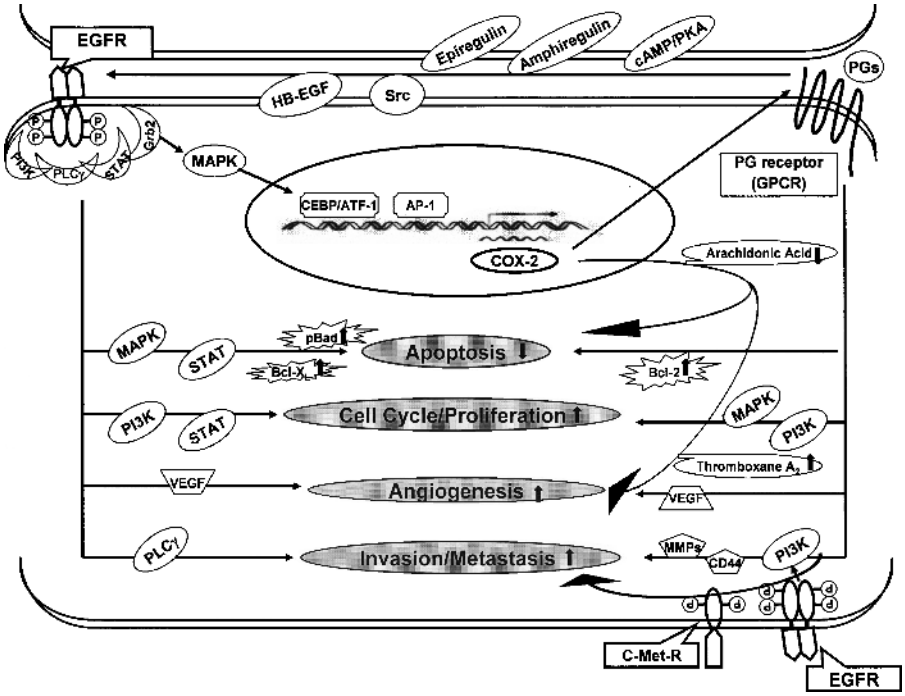

Figure 1. Interaction between the EGFR and COX-2 pathways. Activation of EGFR induces COX-2 mainly via the mitogen-activated protein kinase pathway. On the other hand, prostaglandins, a product of COX-2, can stimulate EGFR activation. Both EGFR and COX-2 pathways act in common on several aspects of carcinogenesis.

receptor mediates the transactivation of EGFR has not been clearly defined but the release of EGFR ligand by Src-activated transmembrane metalloproteinase has been suggested (31, 57). Consistent with these findings, Pai et al. (56) observed that PGE_2-mediated transactivation of EGFR also involved transforming growth factor-α, likely released by Src-activated metalloproteinase. Complicating these findings, Buchanan et al. (58) reported that the transactivation of EGFR by PGE_2 occurred via an intracellular Src-mediated event but not through the release of an extracellular epidermal growth factor-like ligand in colon cancer cell lines. On the other hand, Shao et al. (34) showed that PGE_2 activated EGFR through the induction of increased amphiregulin expression, one of the EGFR ligands. They showed that PGE_2 activated the cyclic AMP/protein kinase A pathway, which induced expression of amphiregulin in a colon cancer cell line. Induction of EGFR expression by overexpression of COX-1 or COX-2 was reported by Kinoshita et al. (55) in a human colon cancer cell line. There is a recent evidence for EGFR and proliferator-activated receptor transactivation through an Src-dependent pathway (59).

A direct collaborative effect between PGE_2 and EGFR on tumor cell phenotypes, such as invasion and proliferation, is also well documented. Shao et al. showed this collaboration between COX-2/PGE_2 and EGFR pathways (34, 60), observing synergistic induction of amphiregulin by PGE_2 and transforming growth factor-α (34). In a follow-up study, they showed that activation of both PGE_2 and EGFR signaling pathways synergistically promoted the growth and migration of colon cancer cells (60). Pai et al. (61) reported that PGE_2 enhancement of invasiveness in a colon cancer cell line was mediated by transactivation of c-Met-R (hepatocyte growth factor receptor), partly through the transactivation of EGFR. Buchanan et al. (58) made a similar observation, showing that PGE_2-induced cell migration was mediated by the transactivation of EGFR, and associated with intracellular Src activation in colon cancer cells.

It is worth noting that COX inhibitor repressed the EGFR-related pathway and in turn, EGFR inhibitor repressed COX-2 expression, indirectly suggesting their interaction (62, 63). COX inhibitors were reported to block the cell proliferation induced by epidermal growth factor in NIH 3T3 cells, which could be reversed by adding exogenous PGE_2 (62). Gefitinib, an EGFR inhibitor, showed inhibition of COX-2 expression in a HNSCC cell line (63). In addition, celecoxib, a selective COX-2 inhibitor, showed a protective effect against HER-2/neu-induced experimental breast cancer, indirectly suggesting a relationship between the epithelial growth factor receptor family and COX-2 (64).

On top of their known interactions, both the EGFR and COX-2 pathways affect the same aspects of carcinogenesis, such as inhibition of apoptosis and induction of angiogenesis. The conclusion that the EGFR and COX-2 pathways directly or indirectly collaborate in pertinent carcinogenic pathways seems justified.

Therapeutic Implication of Targeting COX-2 and EGFR-Mediated Pathways in Chemoprevention

COX-2 as a Target of Therapeutic and Chemopreventive Agents

Selective COX-2 inhibitors have been developed to avoid interrupting the biosynthesis of prostaglandins by COX-1. Studies in preclinical models have clearly showed that COX-2 inhibitors repressed tumor growth (65). Furthermore, COX inhibitors reduce tumor cell migration and tumor invasiveness as well as inhibit angiogenesis in various cell lines and in a xenograft animal model (66–69). These inhibitory effects have been reported in HNC as well as by *in vivo* and *in vitro* experiments (70–74). In terms of a chemopreventive effect in HNC, Wang et al. (75) reported a significant delay of tumor cell growth and reduced angiogenesis using a COX-2 inhibitor, celecoxib, in a xenograft mouse model. In a head and neck carcinogenesis model, Shiotani et al. (76) pretreated rats with a carcinogen, 4-nitroquinoline-1-oxide, followed by a selective COX-2 inhibitor, nimesulide, at the postinitiation stage. They found that the ingestion of carcinogen induced COX-2 expression in premalignant tongue lesions and squamous cell carcinoma. Subsequent COX-2 inhibitor treatment significantly reduced the development of invasive squamous cell carcinoma. The antitumorigenic properties of these inhibitors are both COX dependent and independent (77, 78).

Based on these promising results, many clinical trials of chemoprevention using various COX-2 inhibitors have been conducted in various organs, although mostly in colon. The first chemopreventive trial using a selective COX-2 inhibitor in humans was conducted on familial adenomatous polyposis patients. Familial adenomatous polyposis patients were treated with celecoxib 400 mg bid for 6 months and were compared with a placebo-treated group for their polyp burden. A significant reduction in polyp burden was observed in the celecoxib-treated group (21). Observing the data from this study, the Food and Drug Administration approved celecoxib as an adjuvant therapy for familial adenomatous polyposis patients. Currently, phase II clinical trials are under way to evaluate COX-2 inhibitors for the prevention of recurrence or development of second primary tumor in early-stage HNC patients and the prevention of cancer in patients with oral leukoplakia or dysplasia, using celecoxib.

However, notable cardiovascular toxicity was reported for specific COX-2 inhibitors recently, which resulted in reevaluation of the clinical use of COX-2 inhibitors (79).[3] Studies of COX-2–specific inhibitors, including celecoxib, showed that COX-2 inhibitors increased the thromboembolic cardiovascular risks. A hypothetical explanation is

[3] Department of Health and Human Services. NIH halts use of COX-2 inhibitor in large cancer prevention trial. NIH News, December 17, 2004. Available at http://www.nih.gov/news/pr/dec2004.

Table 1. Preclinical and clinical studies of combined therapy using COX and EGFR inhibitors

Author (reference)	COX inhibitor	EGFR inhibitor	*In vitro/in vivo*/clinical
Tortora et al. (94)	SC-236	ZD1839 (plus protein kinase A antisense)	*In vitro/vivo*
Chen et al. (95)	Celecoxib	ZD1839	*In vitro*
Zhang et al. (96)	Celecoxib	ZD1839	*In vivo*
Luca et al. (97)	Refecoxib	ZD1839	*In vitro*
Torrance et al. (98)	Sulindac	EKB-569	*In vivo*
Krysan et al. (102)	Celecoxib	OSI-774	*In vitro*
Reckamp et al. (100)	Celecoxib	ZD1839	Clinical
Writh et al. (101)	Celecoxib	ZD1839	Clinical
Dannenberg et al. (28)	Celecoxib	EKB-569	Clinical*
Choe et al. (this review)	Celecoxib	OSI-774	Clinical*

*HNSCC chemoprevention trials.

that, unlike nonselective nonsteroidal anti-inflammatory drugs, COX-2 inhibitors could not inhibit platelet aggregation (80, 81). Due to concerns about cardiovascular complications associated with long-term use of COX-2 inhibitors, in December 2004, the National Cancer Institute announced the early cessation of a large colorectal cancer prevention clinical trial with celecoxib.[3] But in April 2005, the Food and Drug Administration announced restricted use of nonsteroidal anti-inflammatory drugs, including celecoxib, by providing revised labels to include more specific information about the potential cardiovascular and gastrointestinal risks.[4]

EGFR as a Target of Therapeutic or Chemopreventive Agents

A variety of strategies have been developed to block EGFR specifically, including monoclonal antibodies, tyrosine kinase inhibitors, ligand-linked immunotoxins, and antisense approaches (37, 82, 83). Among those strategies, monoclonal antibodies, such as IMC-225 (Cetuximab), and tyrosine kinase inhibitors, such as ZD1839 (Iressa or Gefitinib), and OSI-774 (Tarceva or Erlotinib) have shown promising efficacy and are currently being used in clinical studies singly or in combination with other chemotherapeutic agents or radiotherapy. The antineoplastic effects of EGFR inhibitors include inhibition of cell cycle progression, induction of apoptosis, inhibition of angiogenesis, and decreasing metastasis (40, 84). In HNC, EGFR inhibition also showed growth inhibition and inhibition of metastasis in *in vitro* and *in vivo* experiments (84–89).

Like other anticancer agents, EGFR inhibitor toxicity was also reported. The main toxic effects of EGFR tyrosine kinase inhibitors, such as OSI-774 and ZD1839, include headache, diarrhea, and skin rash (22, 90, 91). Rare association with interstitial lung disease was also observed using ZD1839 (92, 93).

[4] The Food and Drug Administration announces series of changes to the class of marketed nonsteroidal anti-inflammatory drugs. Food and Drug Administration News, April 7, 2005. Available at http://www.fda.gov/bbs/topics/news/2005.

Combined Chemopreventive Therapy Using COX-2 and EGFR Inhibitors

Because the underlying genetic mechanisms of many malignant neoplasms are possibly multipath processes combined with complex cross-talk between pathways, specific blocking of single molecular targets would not outwit the variability and complexity of genetic alterations in cancer. Combined treatments using appropriate multiagents may be more effective than single agent treatments. In terms of chemoprevention, combining low doses of drugs too toxic for single use may result in negligible toxicity while eluding drug resistance, resulting in an elegant strategy that is both effective and safe.

Considering the tight connection of the COX-2 and EGFR pathways, the combination of their particular inhibitors may block both pathways in a synergistic or additive manner. This idea has been supported by *in vitro* and *in vivo* studies combining COX-2 and EGFR inhibitors (Table 1). Tortora et al. (94) showed a supra-additive inhibitory effect on tumor growth and angiogenesis by combined treatment with SC-236 (COX-2 inhibitor), ZD1839, and protein kinase A antisense in human colon and breast cancer cell lines and a colon cancer xenograft. We also observed a synergistic growth-inhibitory effect by combining celecoxib and ZD1839 in HNSCC *in vitro* (95) and *in vivo* (96). The combination of celecoxib and ZD1839 augmented G_1 cell cycle arrest and further suppressed phosphorylation of EGFR downstream signaling molecules, such as EGFR, extracellular signal regulated kinase, and AKT. This synergistic growth inhibitory effect was also observed in a breast cancer cell line. De Luca et al. (97) found a synergistic growth inhibitory effect on breast cancer cell lines with a combination of rofecoxib and ZD1839. As with our observation, this effect was associated with significant further inactivation of AKT and extracellular signal regulated kinase.

A synergistic growth inhibitory effect also has been achieved in chemoprevention models. Torrance et al. (98) showed that a combination of sulindac, an inhibitor of both COX-1 and COX-2, and EKB-569, an EGFR tyrosine kinase inhibitor, protected $APC^{Min/+}$ mice remarkably

from the development of intestinal neoplasia, compared with the use of single agents alone. Although 100% of the untreated $APC^{Min/+}$ mice developed ~20 polyps, nearly half the mice treated with both agents developed no polyps at all. In HNC, we observed that pretreatment with celecoxib and ZD1839 before tumor injection results in significant tumor growth delay and inhibition compared with control and single-drug–treated groups (96). The same effect could usually be achieved by at least more than double dose of ZD1839 alone (50 mg/kg in the combination versus 150 mg/kg as a single drug; refs. 96, 99). Combining COX-2 and EGFR inhibitors seems to impede or avert several carcinogenic steps with lower toxicity. As observed previously, cellular proliferation is also more efficiently repressed by this combinational strategy. It may inhibit further progression of genetic instability and make abnormal cells more vulnerable to apoptotic cell death. Synergistic inhibition of angiogenesis has also been achieved. It is rational to expect that inhibiting both pathways with a low dose of each inhibitor would reduce toxicity, because the toxicity from COX-2 inhibitor and EGFR inhibitor have unrelated mechanisms.

Based on preclinical studies, several clinical trials using a combination of COX-2 and EGFR inhibitors are either ongoing or pending activation. Two phase I trials using COX-2 and EGFR tyrosine kinase inhibitors were reported in the American Society of Clinical Oncology meetings of last year and this year (100, 101). The first trial enrolled 12 patients with advanced metastatic and recurrent HNSCC (100). Three of nine evaluable patients showed partial response with no dose-limiting toxicities up to the reporting date. The second trial involved 15 patients with advanced NSCLC (101). The combined drugs were well tolerated. Four of 12 evaluable patients showed partial response and three showed stable disease with no unanticipated toxicities. At present, a randomized multicenter trial using celecoxib and EKB-569 (EGFR tyrosine kinase inhibitor) for preventing oral cancer in patients with oral leukoplakia is pending at the M. D. Anderson Cancer Center (28). We are also planning phase I/II chemopreventive clinical trials using a combination of OSI-774 (Tarceva) and celecoxib for former smokers with premalignant lesions and patients with early stage (stage I/II) HNSCC to prevent HNSCC and second primary and recurrent tumors, respectively. Once launched, these clinical trials will provide valuable information on toxicity, response rate, and appropriate biomarkers for using the combination of COX-2 and EGFR inhibitors in cancer clinics.

Future Prospects and Conclusions

In spite of extensive research in EGFR and COX-2 inhibitors, their combined use for chemoprevention is still in its developmental stage. Further investigations are indeed necessary to develop appropriate chemopreventive strategies in HNC. First, the mechanism by which EGFR and COX-2 pathways are deregulated, interact with each other, or contribute to head and neck carcinogenesis must be clarified. Second, more preclinical studies are critical to evaluate the effectiveness of these combinations and to understand their mechanisms of action as single agents and in combination in HNC specifically. The generation of a good animal model for HNC chemoprevention would be very helpful. Possible drug toxicity from combined use must be evaluated over the long term, although low doses of each drug will be used. Also, substantial biomarkers that can reflect the effect of COX-2 and EGFR combinations need to be identified. Eventually, we may hope to identify the patient population who would derive the most benefit from this combinational chemopreventive approach.

In conclusion, an expanding body of evidence shows tight interaction between the EGFR and COX-2 pathways, which may provide a target for a synergistic inhibitory effect on cancer cell growth using EGFR and COX-2 inhibitors in combination. This combination approach is a promising novel strategy for the chemoprevention of HNC, which may achieve more effective cancer prevention with less drug toxicity or drug resistance than monotherapy with either drug.

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