EFFECTS OF p53 OR p27 OVEREXPRESSION ON CYCLOOXYGENASE-2 GENE EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA CELL LINES

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Abstract: Background. Although cyclooxygenase-2 (COX-2) has been suggested to play an important role in carcinogenesis, the effects of tumor suppressors on COX-2 gene expression and the combined antitumor effects of tumor suppressors and COX-2 inhibitors have rarely been investigated.

Methods. The effects of p53 or p27 gene transfer on COX-2 expression by adenoviral vector and the combined effects of p53 or p27 gene transfer and COX-2 inhibitor exposure on the proliferation of cancer cells were investigated in head and neck squamous cell carcinoma (HNSCC) cell lines.

Results. Overexpression of p53 markedly downregulated the transcription of COX-2, but the overexpression of p27 did not affect COX-2 levels in HNSCC cell lines. The combined antitumor effects of p53 or p27 gene transfer and of a COX-2 inhibitor (NS 398) were mainly at least additive in terms of inhibition of cell proliferation and cell cycle arrest and additive in terms of apoptotic induction.

Conclusions. These results suggest that the overexpression of p53 could exert antitumor effects, at least in part, through the suppression of COX-2 gene expression, whereas growth suppression by the overexpression of p27 probably occurs by mechanisms other than the downregulation of COX-2 expression. In addition, the administration of COX-2 inhibitors, as an adjunct to p53 or p27 gene therapy, could offer a new strategy of cancer treatment and prevention. © 2004 Wiley Periodicals, Inc.

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Cyclooxygenase (COX) enzymes catalyze the rate-limiting step in arachidonic acid metabolism, resulting in the production of prostaglandin, prostacyclin, and thromboxanes. Two COX isoforms have been identified: COX-1 and COX-2.1 COX-1 is expressed constitutively in most cells, where it is involved in the maintenance of tissue
homeostasis. In contrast, COX-2 is normally absent in intact cells but is induced in response to inflammation. 

Recent evidence indicates that COX-2 may represent a novel target for the prevention and treatment of various cancers, including head and neck squamous cell carcinoma (HNSCC). The up-regulation of COX-2 has been detected in several different types of human cancer, and epide-miologic, clinical, and in vitro studies have indicated that the inhibition of COX-2 could be useful for the prophylaxis and treatment of cancers.

COX-2 is up-regulated in response to growth factors, tumor promoters, and cytokines, and several oncogenes, including v-src, v-Ha-ras, HER-2/neu, and Wnt genes, have been reported to enhance the expression of COX-2. In contrast to oncogenes, the effects of tumor suppressor genes (eg, p53 or p27) on COX-2 have rarely been studied, especially in intact cell systems. P53 is known to be important in the suppression of cellular growth and transformation. The induction of wild-type p53 caused cells to undergo apoptosis, and the inactivation of p53 led to the deregulation of the cell cycle and DNA replication, selective growth advantage, and tumor formation. Significantly, p53 is known to either increase or suppress the expressions of a number of target genes.

p27 is one of the cyclin-dependent kinase inhibitors (CKI) and exerts its inhibitory effects on multiple steps of the cell cycle. The main inhibitory action of p27 arises from its binding with cyclin E–cyclin-dependent kinase (CDK)2. In addition to its role as a CKI, p27 is a putative tumor suppressor gene. The expression of p27 protein decreases during tumor development, even though p27 gene mutations are rarely found in cancer. In addition, decreased p27 expression is associated with poor prognosis in several tumors, including those of breast and lung cancer, and the overexpression of p27 may have an antitumor effect in HNSCC cell lines.

Some reports have been published on the effects of p53 on COX-2 gene expression. But those findings were from genetically engineered mouse embryo fibroblasts or resected tumor specimens. Few reports have been published on the effects of tumor suppressor genes, such as p53 or p27, on COX-2 gene expression in intact cancer cells, especially in HNSCC cells. In this work, we constructed a recombinant adenovirus-p53 (ad-p53) and an adenovirus-p27 (ad-p27) and investigated their effects on COX-2 gene expression in HNSCC cell lines. We then tested their antitumor effects on the inhibition of cell proliferation, the arrest of the cell cycle, and the induction of apoptosis in HNSCC cells. In addition, we investigated the combined effects of p53 or p27 gene transfer and of a specific COX-2 inhibitor on the malignant behaviors of HNSCC cells.

MATERIALS AND METHODS

Cell Lines and Reagents. Three human HNSCC cell lines (SNU-1041, SNU-1066, and SNU-1076) were purchased from the Korean Cell Line Bank (Seoul National University, Seoul, Korea). All cell lines were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco-BRL, Grand Island, NY) supplemented with 10 mg/mL gentamicin and 10% fetal bovine serum (FBS) and maintained at 37°C under 5% CO2 in humidified air. All cell culture consumables were obtained from Gibco BRL, Grand Island, NY, unless otherwise stated. A selective COX-2 inhibitor, N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide (NS-398) (Cayman Chemical Co., Ann Arbor, MI), was prepared as a 10 mM stock solution. The required final concentrations of NS-398 were prepared by diluting aliquots of this stock solution in medium, together with an appropriate volume of dimethyl sulfoxide (DMSO).

Construction of Recombinant ad-p53 and ad-p27. Wild-type ad-p53 and ad-p27 were constructed in our laboratory as reported previously. Briefly, the cDNAs of human p53 and p27 (kindly provided by J. Massague, Memorial Sloan-Kettering Cancer Center, New York, NY) were subcloned into the KpnI and BamHI sites of the polylinker of adenoviral shuttle vector, pAC CMV pLpA (kindly provided by Robert Gerard, The University of Texas Southwestern Medical Center, Dallas, TX), respectively. The resulting pAC CMV-27 and pAC CMV-53 were cotransfected, respectively, with pJM17 (also kindly provided by R. Gerard) into HEK293 cells (human renal embryonal cells immortalized by stable transfection with E1 of adenovirus; purchased from the Korean Cell Line Bank, Seoul, Korea), using the standard calcium phosphate precipitation method. HEK293 cells were maintained in Dulbecco’s modified Eagle’ medium (DMEM) (Gibco-BRL, Grand Island, NY) containing 2% FBS until the onset of a cytopathic effect. The generating adenoviruses were purified three times by plaque assay and confirmed by DNA se-
quencing of the viral DNA and by Western blotting. A large-scale stock of adenoviruses was prepared by the standard CsCl method. Recombinant adenovirus without any therapeutic gene (ad-null) was used as the control virus in all experiments.

Transfection of Cell Lines with ad-p27 and ad-p53. Exponential growing HNSCC cells were transfected with ad-p27 (100 MOI) and ad-p53 (100 MOI) for 90 minutes with gentle frequent shaking and then incubated with complete media for the experiment.

Western Blot Analysis. HNSCC cells were transfected with 100 MOI of ad-p53 or ad-p27 (or ad-null for the control virus) for 90 minutes and then grown for 48 hours in complete media. Western blotting for p53, p27, COX-2, and COX-1 was performed with the electrochemiluminescence (ECL) Western blotting system (Amersham, England). Monoclonal mouse anti-human p53 antibody (1:1000, Santa Cruz Biotech, Santa Cruz, CA), polyclonal rabbit anti-human p27 antibody (1:1000, Santa Cruz Biotech), and monoclonal mouse anti-human COX-2 and COX-1 antibodies (1:1000, Transduction Laboratories, Lexington, KY) were used as the primary antibodies for p53, p27, COX-2, and COX-1, respectively.

Cell Proliferation Assay. Each of the three HNSCC cell lines was plated in 96-well plates (5 × 10^3/well). After 12 hours of incubation, cells were transfected with 100 MOI of ad-null, ad-p53, or ad-p27 for 90 minutes and then incubated with complete media. Cells of the control group were incubated with serum-free media for 90 minutes and then regrown in complete media. Cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay every other day.

To evaluate the combined effects of p53 or p27 gene transfer and NS-398 exposure on the proliferation of the targeted cell lines, at day 1 after transfection of ad-p53 or ad-p27 into the three HNSCC cells, old medium was replaced with fresh medium containing NS-398 at concentrations of 20 or 40 μM, or an equivalent volume of DMSO. Then at days 3 and 5, an MTT assay was performed.

Cell Cycle Analysis. HNSCC cell lines were transfected with 100 MOI of ad-null, ad-p53, or ad-p27 for 90 minutes and grown for 48 hours in complete media. Cell cycle alterations were measured by flow cytometry with use of the Cycle

![Figure 1](image1.png)

**FIGURE 1.** Western blotting for p27, p53, and cyclooxygenases in head and neck squamous cell carcinoma (HNSCC) cell lines. Western blot analyses for p27, p53, and cyclooxygenases were carried out 48 hours after transfection with 100 MOI of ad-p27 or ad-p53 (Ad-null, untransduced). The results demonstrated that p27 did not affect the levels of cyclooxygenase (COX)-2 and COX-1 despite its overexpression after transfection with ad-p27 compared with ad-null in all HNSCC cell lines tested. In the case of p53, it was overexpressed after transfecting ad-p23 into all three HNSCC cell lines, and this was accompanied by the nearly complete suppression of COX-2 expression compared with ad-null in all HNSCC cell lines tested. However, the overexpression of p53 did not affect the levels of COX-1 versus ad-null.

![Figure 2](image2.png)

**FIGURE 2.** Quantitative image analysis of the Western blotting bands. When p27 overexpressed, compared with ad-null, a certain settled pattern of change in total optical densities of the bands for cyclooxygenase (COX)-1 or COX-2 was not observed in three head and neck squamous cell carcinoma (HNSCC) cell lines tested. But in case of p53, compared with ad-null, total optical densities of the bands for COX-2 were consistently decreased by approximately 90% or more in all three HNSCC cell lines tested.
Test plus kit protocol (Becton Dickinson, San Jose, CA).

To evaluate the combined effects of p53 or p27 gene transfer and NS-398 exposure on the cell cycle parameters of the targeted cell lines 1 day after transfection with ad-p53 or ad-p27 into the three HNSCC cells, old medium was replaced with fresh medium containing NS-398 at concentrations of 20 or 40 μM or an equivalent volume of DMSO. Then, at day 3 after the transfection, cell cycle analysis was performed, as described previously.

**Quantification of Apoptosis.** Flow cytometric analysis of annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI)–stained cells was performed with an Annexin V apoptosis kit (PharMingen, San Diego, CA), as recommended by the manufacturer. Briefly, HNSCC cells were transfected with 100 MOI of ad-null, ad-p53, or ad-p27 for 90 minutes and grown for 48 hours in complete media. Cells were then detached by a brief trypsin treatment, stained with annexin V and PI, and sorted by FACSstar flow cytometry (Becton Dickinson). The proportions showing early apoptosis (positive for annexin V and negative for PI) were determined.

To evaluate the combined effects of p53 or p27 gene transfer and NS-398 exposure on the apoptosis of targeted cell lines 1 day after transfection with ad-p53 or ad-p27 into the three HNSCC cells, old medium was replaced with fresh medium containing NS-398 at concentrations of 20 or 40 μM or an equivalent volume of DMSO. Then, 3 days after the transfection, apoptosis was determined by FACS as described previously.

**Transfection of COX-2 Promoter and Luciferase Assay.** SNU-1041 cells were cotransfected with ad-p53 at various MOIs and with luciferase expression vector containing COX-2 promoter

![Graphs showing growth curves of HNSCC cells transfected with ad-p27 or ad-p53](image)

**FIGURE 3.** Growth curves of head and neck squamous cell carcinoma (HNSCC) cells after transfecting with ad-p27 or ad-p53. After transfecting cells with 100 MOI of ad-p27 or ad-p53 (Ad-null, untransduced), cell proliferation was determined by MTT assay every other day. A statistically significant inhibition of proliferation was observed in all cell lines transfected with ad-p27 or ad-p53 versus cells transfected with ad-null (*, one-way analysis of variance [ANOVA], \( p < 0.05 \)). OD, optical density (OD540).
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Statistical Analysis. Statistical evaluations were performed using one-way analysis of variance (ANOVA), repeated measures ANOVA, and Tukey’s Studentized Range test, where appropriate, with a level of significance at \( p < .05 \). Graphically, data are shown schematically with error bars and represent plots of means ± standard error of the mean (SEM) (SigmaPlot Scientific Software; SPSS Inc., Chicago, IL).

RESULTS

Western Blot Analysis. Forty-eight hours after transfecting with 100 MOI of ad-p53 or ad-p27 into HNSCC cell lines, the expression of p53, p27, COX-2, and COX-1 were measured by Western blotting. Figure 1 shows that the expression of p53 increased after ad-p53 transfection and that this was accompanied by the near complete suppression of COX-2 expression in all HNSCC cell lines tested, whereas p27 did not affect the levels of COX-2 despite its overexpression after ad-p27 transfection. As a control, Western blotting of the cell lysate protein for COX-1 was also carried out, and overexpressions of p53 or p27 were found not to affect the levels of COX-1. To confirm the findings, the procedure was repeated for the same HNSCC cell lines, and to express the results numerically, quantitative image analysis was made for the bands obtained from two different Western blottings. When p27 overexpressed, compared with ad-null, a certain settled pattern of change in total optical densities of the bands for COX-2 was not observed in three HNSCC cell lines tested. But in case of p53, compared with ad-null, total optical densities of the bands for COX-2 were consistently decreased by approximately 90% or more in all three HNSCC cell lines tested (Figure 2).

Effects of p53 and p27 on HNSCC Cell Growth. Transfection with ad-p27 or ad-p53 induced marked growth suppression compared with cells untransduced or transduced with ad-null in all three HNSCC cells (Figure 3).

Cell Cycle Analysis in HNSCC Cells. P53 or p27 gene transfer into HNSCC cells induced significant increases in the percentages of cells in the G0/G1 phases and concomitant reductions in the number of cells at the S phase versus transfected with ad-null cells in all three cell lines, suggesting G0/G1 arrest. Representative percentages of cells in each phase of the cell cycle 48 hours after transfection of the respective genes are presented in Table 1.

Induction of Apoptosis in HNSCC Cells. Results are expressed as the percentage of cells positive for annexin V and negative for PI, which represent early apoptotic cell fractions 48 hours after transfection (Table 2). P53 gene transfer into HNSCC cells induced significant increases in the percentages of early apoptotic cells compared

| Table 1. Cell cycle analysis after transfection with ad-p27 or ad-p53 into the SNU 1066 cell line. |
|-----------------------------------------|-----------------|-----------------|-----------------|
| Group       | G0/G1 (%) | S (%)         | G2/M (%)         |
| Control     | 45.77 ± 7.48 | 37.57 ± 8.69 | 16.66 ± 7.30 |
| Ad-null     | 44.74 ± 3.11 | 41.02 ± 8.51 | 14.26 ± 5.35 |
| Ad-p27     | 54.68 ± 8.31| 29.06 ± 4.37 | 16.26 ± 5.71 |
| Ad-p53     | 62.49 ± 6.01| 26.77 ± 5.15 | 10.74 ± 3.40 |

Note: Data are expressed as the mean (± SEM); the percentage of cells in given stages of the cell cycle phase is quoted at 48 hours (n = 6).

| Table 2. Early apoptotic cell fractions after transfection with ad-p27 or ad-p53 in HNSCC cell lines. |
|-----------------------------------------|-----------------|-----------------|
| Cell line     | Early apoptotic cell fraction (%) |
| SNU-1041     | Control 11.08 ± 5.48 |
|              | Ad-null 10.31 ± 4.70 |
|              | Ad-p27 10.30 ± 4.06 |
|              | Ad-p53 26.49 ± 8.08 |
| SNU-1066     | Control 10.08 ± 3.34 |
|              | Ad-null 8.85 ± 4.76 |
|              | Ad-p27 18.81 ± 4.44 |
|              | Ad-p53 33.01 ± 6.23 |
| SNU-1076     | Control 11.68 ± 3.12 |
|              | Ad-null 11.15 ± 2.59 |
|              | Ad-p27 17.33 ± 4.73 |
|              | Ad-p53 33.85 ± 5.32 |

Abbreviation: HNSCC, head and neck squamous cell carcinoma.

Note: Data are expressed as mean (± SEM); percentages represent cells positive for annexin V and negative for PI at 48 hours (n = 6).

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with cells transduced with ad-null in all three cell lines. p27 gene transfer also induced significant apoptosis in SNU-1066 and SNU-1076 cell lines but not in the SNU-1041 cell line.

COX-2 Promoter Assay. To confirm the effects of p53 on the expression of the COX-2 gene in HNSCC cells, luciferase activity was measured 48 hours after cotransfecting with a luciferase expression vector containing the COX-2 promoter and ad-p53 at various MOIs into the SNU-1041 cell line. Figure 4 shows the outcome of luciferase assay; results are expressed as ratios relative to the control. p53 gene transfer into SNU-1041 cells significantly suppressed the transcription of COX-2 promoter at all MOIs of ad-p53 transfected compared with ad-null, and the extent of suppression was dependent on the MOI of ad-p53. In cases of the SNU-1066 and SNU-1076 cells, viable HNSCC cells were rarely observed 48 hours after cotransfecting ad-p53 and the COX-2 promoter by cationic liposome–enhanced gene transfer.

Combined Effects of p53 or p27 Gene Transfer and NS-398 Exposure. We evaluated the combined effects of p53 or p27 gene transfer and NS-398 exposure on cell proliferation, cell cycle parameters, and the apoptosis of the targeted cell lines. At day 1 after transfection, infected cells were exposed to NS-398 at concentrations of 20 or 40 \( \mu M \), or an equivalent volume of DMSO. Cell cycle analysis and FACS analysis for apoptosis
were performed at day 3, and MTT cell proliferation assays were done at days 3 and 5.

The results of the cell proliferation assay in the three HNSCC cell lines tested are illustrated in Figure 5 (data at day 5 are not shown). NS-398 treatment resulted in the dose-dependent inhibition of cell growth when ad-null was transfected. When ad-p27 or ad-p53 was transfected, the combined effects of gene transfer and NS-398 exposure on cell growth inhibition were found to be mostly at least additive compared with ad-null. The combined effects of gene transfer and NS-398 exposure on G0/G1 arrest and S phase reduction were also mainly at least additive compared with ad-null for both p27 and p53. However, their combined effects on the induction of apoptosis were additive in all cell lines tested compared with ad-null for both p27 and p53. Representative data for their combined effects on cell cycle arrest and apoptotic induction are illustrated in Figure 6.

DISCUSSION
It seems that overexpression of COX-2 contributes to cancer growth by inhibiting apoptosis through the synthesis of antiapoptotic protein (bcl-2), the sequestration of nucleobindin, or the decreased production of ceramide. Moreover,

FIGURE 6. Combined effects of p27 or p53 gene transfer and NS-398 exposure on cell cycle arrest and the induction of apoptosis in the SNU-1066 cell line (measured by FACS analyses 3 days after transfection). At day 1 after transfecting with 100 MOI of ad-p53 or ad-p27 (Ad-null, untransduced), the infected cells were exposed to NS-398 at concentrations of 20 or 40 μM or an equivalent volume of dimethyl sulfoxide. The combined effects of gene transfer and NS-398 exposure in G0/G1 arrest and S phase reduction were mainly at least additive compared with ad-null in both p27 and p53. However, their combined effects on the induction of apoptosis were additive, versus ad-null in both p27 and p53 transfected cells. *, At least additive effect compared with ad-null (Tukey’s Studentized Range test, p < .05); †, additive effect compared with ad-null (Tukey’s Studentized Range test, p > .05).
it seems quite possible that COX-2 protein contributes to cancer cell proliferation by inducing genes involved in cell proliferation and the delay of G1 cell cycle progression.25,26 In addition, COX-2–induced tumor invasion and metastatic spread are thought to be associated with the enhanced synthesis of prostaglandin E₂, interleukin-6, haptoglobin, the reduced expression of cadherin, and prostaglandin-mediated immune suppression.27–29

The repression of the transcription of a number of target genes by p53 is thought to be important for p53-mediated apoptosis and for the suppression of cellular growth and transformation of cancer cells. However, the exact mechanisms underlying these effects are not clearly understood.30 On the other hand, the overexpression of COX-2 in intestinal epithelial cells and cancer cells is known to inhibit apoptosis and to enhance cell proliferation, tumor metastases, and angiogenesis by the mechanisms mentioned previously. The results observed in our study show that p53 downregulates the transcription of COX-2 and concomitantly induces significant apoptosis, cell cycle arrest, and the inhibition of cell proliferation, at least in part, through the suppression of COX-2 gene expression. The finding that p53 suppresses the transcription of COX-2 could be important in the understanding of p53–mediated apoptosis, cell cycle arrest, and the inhibition of cell proliferation in cancer cells, especially in HNSCC.

It is known that p53 suppresses a variety of promoters that contain tumor-associated transplantation antigen (TATA) elements.31 This suppression is thought to occur through its direct interaction with components of the basal transcription machinery, like TATA binding protein (TBP). In some studies, p53 was found to inhibit the binding of TBP to several promoters, most probably through protein–protein interactions.31,32 Under such a circumstance, TBP is unable to assemble a functional transcription initiation complex. In this study, p53 inhibited COX-2 gene expression. One possible explanation for this result is that p53 and TBP compete directly for the TATA binding site. A recent in vitro experiment in a cell-free system demonstrated that p53 inhibits the formation of the complex formed between TBP and COX-2 promoters.20

Regarding the relation between cell cycle–related genes and COX-2, only a few studies have described the effects of COX-2 inhibitors on the regulation of cell cycle–related gene expression. Goldberg et al33 demonstrated that sulindac increased the expression of p21WAF1 in human colon cancer cells, and Hung et al34 reported that NS-398 upregulated p27 expression by means of posttranslational control in human lung cancer cells. However, no study has described the effect of cell cycle–related genes, such as p27, on the expression of COX-2. In this study, we provide the first evidence that the expression of COX-2 may not be affected by the overexpression of p27 and that p27 may exert its anticancer action through mechanisms other than the suppression of COX-2 expression in HNSCC cell lines.

COX-2 inhibitors are known to inhibit cancer cell growth by increasing apoptosis and cell cycle arrest.5,35 However, the enzymatic inhibitors of COX-2, such as nonsteroidal anti-inflammatory drugs (NSAIDs), have limitations in terms of the prevention or treatment of cancer. COX has both cyclooxygenase and peroxidase activities. Aside from being important for prostaglandin synthesis, the peroxidase function may contribute to the activation of procarcinogens. Drugs such as NSAIDs inhibit cyclooxygenase but not the peroxidase function of COX, which potentially limits their effectiveness. In addition, increased transcription of COX-2 by means of a feedback mechanism could result in the synthesis of functional enzyme despite drug therapy. However, p53 can directly suppress the transcription of the COX-2 gene, as shown by this study, by interfering with the signaling mechanisms responsible for the upregulation of COX-2. This might be an important advantage in preventing or treating cancer, especially HNSCC. The overexpression of p53 can inhibit carcinogenesis or treat the cancer more effectively than drugs that inhibit the cyclooxygenase activity of COX without having the aforementioned functional limitations. A detailed understanding of the regulation of COX-2 transcription should provide an important insight into possible approaches designed to block the expression of COX-2 in cancer cells, thereby inhibiting all functions of this enzyme.

This study also revealed that the use of NS-398, a specific COX-2 inhibitor, as an adjunct to adenovirus-mediated p53 or p27 gene transfer into HNSCC cells proved to be more successful than gene transfer alone in inducing apoptosis, cell cycle arrest, and the inhibition of cell proliferation in HNSCC cell lines. These data show that the combined effects of NS-398 and the overexpression of p53 or p27 on the induction of apoptosis were additive in all HNSCC cell lines.
tested, and their combined effects on the inhibition of HNSCC cell proliferation and cell cycle arrest were mainly at least additive. This study demonstrates that the anticancer actions of p53 or p27 are potentiated by NS-398 in HNSCC cell lines. These results suggest that the adjunctive administration of NS-398 with adenovirus-mediated p53 or p27 gene transfer into human cancer cells might be a new clinical strategy for the treatment or prevention of cancers, especially HNSCC.

The findings of our study are of considerable clinical interest, because the administration of COX-2 inhibitors may well be feasible in many cancer patients without serious side effects in view of previously obtained favorable data on its use as an anti-inflammatory drug. With the recent development of highly selective COX-2 inhibitors, such as NS-398, we expect that the use of selective COX-2 inhibitors as adjuncts with various anticancer agents will lead to greater treatment efficacies in high-risk patients without compromising quality of life.

In conclusion, p53 was found to exert its antitumor effects, at least in part, by suppressing COX-2 gene expression in HNSCC cell lines. Considering the importance of COX-2 as a participant in carcinogenesis, these findings provide a new insight into understanding the functioning of p53 and p53-related cancers. This study also provides first evidence that the growth suppression induced by p27 may not occur through the suppression of COX-2 expression in HNSC cell lines. In terms of anticancer mechanisms, NS-398 is believed to use the same target as p53 (ie, COX-2) and that p27 does not. We believe that further in vitro and in vivo animal or clinical studies will facilitate the development of new biologic methods for treating or preventing HNSCC and that the administration of COX-2 inhibitors in combination with p53 or p27 gene therapies offers us a promising candidate strategy.

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