Cyclooxygenase 2 Promoter–Based Replication-Selective Adenoviral Vector for Hypopharyngeal Cancer

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Objective: To explore the potential clinical application of the oncolytic activity of cyclooxygenase 2 (COX-2) promoter–based, conditional, replication-selective adenovirus vector for hypopharyngeal squamous cell carcinoma.

Design: In vivo study and retrospective study.

Setting: Kobe University Hospital, Kobe, Japan.

Subjects: Expression of COX-2 in hypopharyngeal cancers treated at Kobe University Hospital was immuno-histochemically investigated. In addition, nude mice bearing human hypopharyngeal cancer cells (H891) were used to analyze oncolytic activity of a conditional replication-selective adenovirus vector in which the expression of E1a, required for viral replication, is controlled by the COX-2 promoter Ad-COX2-E1a.

Results: In vivo assays showed significant growth suppression in the murine hypopharyngeal model. Cyclooxygenase 2 expression was observed in 75.3% of hypopharyngeal cancers, especially in differentiated tumor cells (P=.001; r=0.433).

Conclusion: In this study, we demonstrated the potential of oncolytic therapy using the COX-2–promoter based, conditional, replication-selective adenovirus for COX-2–expressing hypopharyngeal squamous cell carcinomas.

CELL AND CELL CULTURE

Human hypopharyngeal cancer cell line H891, which was established in the Yokohama City University School of Medicine,6 was used in this study. Cell line H891 was maintained in complete RPMI 1640 Medium (Sigma, St Louis, Missouri) supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37°C in 5% carbon dioxide. Fresh medium was added to the cells twice a week.

CONSTRUCTION AND PRODUCTION OF REPLICATION-COMPETENT ADENOVIRAL VECTOR Ad-COX2-E1a

The details of the production of the COX-2–based replication-selective adenoviral vector were described in our previous report. Briefly, the COX2-E1a promoter was ligated to the pXC1, which possesses the adenovirus sequences from base pairs 22 to 5790 containing the E1 gene (Microbix Biosystems Inc, Toronto, Ontario, Canada)7 to obtain pXC1-COX2-E1a. The recombinant Ad-COX2-E1a virus was prepared by means of homologous recombination8 using pXC1-COX2-E1a mixed with pBHGE3 plasmid containing Ad5 sequences with a wild-type E3 region and E1 deletion of base pairs 188 to 1339.9

TREATMENT AND MEASUREMENTS

The H891 cells (1 × 10⁶) were suspended in 100 µL of phosphate-buffered saline (PBS) and subcutaneously injected into the backs of male nude mice (5 weeks old and weighing 20-23 g) with a BALB/c (nu/nu) genetic background (CLEA Japan Inc, Tokyo, Japan). When tumors with a 5- to 6-mm diameter had developed, 18 nude mice were randomly separated into 3 groups. Group 1 was given PBS only (n=6); group 2 was given Ad-CMV-βgal. It is noteworthy that the difference gradually increased with time, from P = .05 at day 4; P = .01 at day 6; and P = .006 at day 8; P = .006 at day 11; and P = .006 at day 14 (Figure 3). These findings suggest that the growth inhibitory effect of Ad-COX-2-E1a continues and increases as a result of viral replication.

RELATION BETWEEN COX-2 EXPRESSION AND CLINICOPATHOLOGIC FEATURES

Cyclooxygenase 2 was observed mainly in the tumor area and occasionally in areas of mild to severe dysplasia. Normal epithelium adjacent to the COX-2–positive tumors was not stained. Cyclooxygenase 2 expression was positive in 75.3% and negative in 24.7% of hypopharyngeal
Cancers, and statistical analysis showed a significant difference between the intensity of COX-2 expression and tumor differentiation \((P = 0.001; r = 0.433)\) (Table 1).

The associations between clinicopathological features and COX-2 expression are listed in Table 2. No significant association was observed between COX-2 expression and age, sex, stage, pathologic T stage, or pathologic N stage. Figure 4 shows survival curves of the patients with hypopharyngeal cancer in relation to COX-2 expression. The 5-year survival rates of patients with and without COX-2 expression were 54% and 43%, respectively \((P = 0.21)\).

**COMMENT**

Cyclooxygenase 2, which is primarily responsible for prostaglandins produced at inflammatory sites, is virtually undetectable in most tissues under physiologic conditions. In contrast, recent studies have demonstrated that COX-2 is expressed in several cancer tissues, including head and neck cancers, and may be important for carcinogenesis.\(^5\,^9\,10\,12\,13\) Taking advantage of this tumor-specific expression of COX-2, our research group has recently generated the COX-2 promoter–based replication-selective adenoviral vector Ad-COX-2-E1a and have demonstrated its antitumor effect against COX-2–
expressing hypopharyngeal squamous cell carcinoma in an in vitro study.3

Herein, we show that Ad-COX-2-E1a also significantly inhibited the growth of COX-2–expressing tumors without serious adverse effects in an animal model. In addition, immunohistochemical analysis demonstrated that 75.3% of the hypopharyngeal cancers expressed COX-2, which suggests that about three-fourths of patients with hypopharyngeal cancer are potential candidates for this treatment.

Tumor cell differentiation was significantly related to COX-2 expression, which was upregulated in well-differentiated tumors, suggesting that its expression may be involved in the pathogenesis or growth of well-differentiated tumor cells. Alternatively, COX-2 overexpression in these tumors may be a consequence of squamous differentiation in an abnormal setting. In this regard, it should be noted that COX-2 expression becomes weaker when hypopharyngeal cancer progresses to a more aggressive phenotype, thus becoming less differentiated. Indeed, while statistically not significant, the 5-year survival rate of patients without COX-2 expression was worse than that of the patients with COX-2 expression. Cyclooxygenase 2 expression may therefore indicate a relatively favorable condition, even though the prognosis of patients with COX-2–expressing hypopharyngeal cancer remains unsatisfactory.

In terms of therapeutic strategy, the effects of chemotherapy or radiotherapy are generally less favorable for well-differentiated tumors.14,15 On the other hand, Ad-COX2-E1a is more effective for COX-2–expressing tumors, as evident in this and our group’s previous study.3 Since more than half of the differentiated tumors in this study expressed COX-2, Ad-COX2-E1a may compensate for the chemoresistance and/or radioresistance of well-differentiated cancers.

Adenoviral therapy in combination with chemotherapy has shown promising results in various preclinical models and clinical trials.16 In particular, the treatment regimen of intratumoral d1520 injection in combination with cisplatin and 5-fluorouracil for patients with recurrent head and neck cancer has already entered phase 2 trials and shown promising results.17,18 As reported by many investigators, direct intratumoral injection is more efficient than other delivery methods, eg, intravenous, intra-arterial, or intraperitoneal injection.19-23 Since the head and neck areas are easy to approach, patients with head and neck cancer are thought to be some of the most suitable candidates for adenoviral therapy.

Recently, many studies have reported that selective COX-2 inhibitors are useful in prevention or treating various neoplasms, including head and neck cancer.24-27
effects are related to suppression of cell proliferation and induction of apoptosis, and the expression of COX-2 itself is not suppressed by selective COX-2 inhibitors. Because the effect of Ad-COX2-E1a depends on the expression of COX-2 itself, the combination therapy of Ad-COX2-E1a and selective COX-2-inhibitors may lead to the synergic effects in COX-2-expressing head and neck cancers. Although viral introduction as part of adenoviral therapy is highly efficient, injury to normal organs must be avoided. In this connection, newer generations of adenoviral vector have recently been developed to further restrict viral replication to tumor cells. Further studies would make the implementation of chemoradiotherapy with selectively replicative viruses a reality.

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