Increased Expression of Interleukin-1α and Cyclooxygenase-2 in Human Gastric Cancer: A possible Role in Tumor Progression

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Abstract. Background: Cyclooxygenase (COX)-2 mRNA and protein expression have been found to be frequently up-regulated in human gastric cancers and cell lines. COX-2 is the inducible form of COX, and interleukin (IL)-1α may be one of the stimulators of COX-2. Materials and Methods: The relationship between IL-1α and COX-2 expression was examined in human gastric cancer tissues and cell lines. Results: IL-1α mRNA was expressed in 19 out of 32 human gastric cancer specimens (60%), while it was not expressed in any of the paired normal gastric mucosa specimens. The incidence of IL-1α mRNA expression was significantly higher in patients with pT2-pT4 tumors than in those with pT1 tumors (86% vs. 39%, p<0.05). IL-1α mRNA was expressed in 94% of COX-2-positive tumors, while it was expressed in only 25% of COX-2-negative tumors (p<0.0001). When IL-1α was added to the medium of gastric cancer cell lines (MKN28 and MKN45), it enhanced the expression of IL-1α itself and COX-2 mRNA in both cell lines. Exogenous IL-1α stimulated cancer cell growth in both cell lines, while such growth stimulation was suppressed by anti-IL-1α antibody or IL-1 receptor antagonist. IL-1α-stimulated cell growth was also suppressed by anti-COX-2 antibody. Conclusion: Our data demonstrated that IL-1α and COX-2 mRNA were frequently co-expressed in human gastric cancer tissues, and suggested that the IL-1α-COX-2 pathway might be involved in tumor progression by regulating cancer cell proliferation.

Cyclooxygenase (COX) is a key enzyme in prostaglandin (PG) biosynthesis (1). Two isoforms of COX have been identified, namely constitutively expressed COX-1 and mitogen-inducible COX-2 (2-4). An enhanced expression of COX-2, but not COX-1, has been found in various cancers (5-10) including gastric cancer (11, 12). We previously demonstrated clinical evidence that COX-2 may contribute to tumor progression in human gastric cancers (13, 14). COX-2-mediated PG biosynthesis and COX-2-induced angiogenesis may be involved in the development of cancer (13-16). COX-2 has been reported to be rapidly induced by various stimuli such as mitogens, lipopolysaccharides and cytokines (17, 18).

Interleukin (IL)-1α, which is produced mainly by activated macrophages, mediates local and systemic responses to infection and inflammation (19-21). Recent studies have shown the importance of IL-1α in tumor progression. The expression of IL-1α has been found in thyroid carcinoma (22), ovarian carcinoma (23), lung carcinoma (24) and ATL cells (25). IL-1α has been reported to be expressed in gastric carcinoma as well, and is considered to be associated with the stimulation of tumor proliferation and the facilitation of liver metastasis (26-28). Although IL-1α has been shown to induce COX-2 expression in human umbilical vein endothelial cells (18), the involvement of IL-1α and COX-2 in human cancer tissues remains unclear. In this study, we attempted to determine the relationship between IL-1α and COX-2 mRNA expression in human gastric cancer specimens. Furthermore, the effects of IL-1α on COX-2 expression and cell proliferation in human gastric cancer cell lines were examined.

Materials and Methods

Patients. The expression of IL-1α and COX-2 mRNA was assayed in biopsy specimens by means of reverse transcription-polymerase chain reaction (RT-PCR). After obtaining the patients’ informed consent, biopsy samples were endoscopically obtained from patients at the National Defense Medical College Hospital, Tokorozawa, Japan. Basically, six specimens were taken from each gastric lesion. Of these, three specimens were stored at −80°C for RT-PCR. The remaining specimens were used for histological diagnoses, while samples that contained neoplastic
cellularities of less than 50% of the cells were excluded as subjects for RT-PCR. Thirty-two histologically confirmed gastric adenocarcinomas and their paired normal gastric mucosa specimens, in which 100% of the cells contained non-neoplastic cellularity, were examined. All gastric carcinomas were surgically removed and then histologically evaluated.

RT-PCR for IL-1α and COX-2. RT-PCR was performed as described previously (14). Briefly, total RNA was isolated from the samples and then reverse transcribed. PCR was performed using a thermal cycler. Initial denaturation was done at 94°C for 2 min followed by 30 cycles (94°C for 45 sec, 60°C for 45 sec and 72°C for 2 min) of amplification. The final extension was done at 72°C for 7 min. The PCR products were analyzed by 1.5% agarose gel electrophoresis and visualized with ethidium bromide. The membrane image was incorporated into a computer and quantitated using NIH image software. The primers were as follows: human IL-1α (491-bp), sense 5'-CAAGGAGACATGGTAGTAGCAACCAACG-3', antisense 5'-TAGTGCCGTGAAGGTCCACGT GGAGG-3'; human COX-2 (843-bp), sense 5'-GATTGCCCGACTCCCTTG GTGTC-3', antisense 5'-TCTACCACCAACCGAGG-3'. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

Cell lines and materials. Two human gastric cancer cell lines, MKN28 (well-differentiated adenocarcinoma) and MKN45 (poorly-differentiated adenocarcinoma), were provided by the Health Science Research Resources Bank in Japan. The cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). The reagents used for this study were as follows: recombinant human IL-1α (Genzyme Co., Cambridge, MA, USA), anti-human IL-1α antibody (R & D Systems Inc., Minneapolis, MN, USA), IL-1 receptor antagonist (IL-1ra, R & D), and anti-human COX-2 antibody (Immunobiological Laboratories, Fujioka, Japan).

IL-1α treatment of gastric cancer cells. The MKN28 and MKN45 cells were seeded onto a plate (1x10⁵ cells/well) in RPMI 1640 medium (10% FBS). The cells were grown to 70% confluence and then incubated in RPMI 1640 medium (5% FBS) containing 0, 1 and 10 units/ml of recombinant human IL-1α for 24 h. IL-1α and COX-2 mRNA were detected by RT-PCR.

Measurement of proliferation. The MKN28 and MKN45 cells (1x10⁵ cells/well) were seeded onto plates and cultured for 24 h in RPMI 1640 medium (10% FBS). The cells were then incubated in the absence (control) or presence of recombinant human IL-1α (10 units/ml) in RPMI 1640 medium (5% FBS). In another experiment, the cells in the medium (5% FBS) containing recombinant human IL-1α (10 units/ml) were treated with anti-IL-1α antibody (0.5%), IL-1ra (1 ng/ml), or anti-COX-2 antibody (10 ng/ml). Cell growth was assayed using a cell proliferation assay system (Seikagaku Co., Tokyo, Japan), as described previously (29). The activity of 100 units of recombinant IL-1α was blocked by anti-IL-1α antibody and IL-1ra at concentrations of 0.5% and 0.1 ng/ml, respectively (26).

Statistical analysis. Differences between the groups were analyzed using either the Chi-square test or Student’s t-test. A p value of less than 0.05 was considered statistically significant.

Results

Expression of IL-1α and COX-2 mRNA in human gastric cancer tissues. IL-1α mRNA was expressed in 19 out of 32 human gastric cancer specimens (60%), while it was not
expressed in any of the paired normal gastric mucosa specimens (Figure 1). The incidence of IL-1α mRNA expression was significantly higher in patients with pT2-pT4 tumors than in those with pT1 tumors ($p < 0.01$). IL-1α mRNA expression was not associated with lymph node involvement or histological type (Table I).

COX-2 mRNA was expressed in 16 out of 32 cases (50%), while it was not expressed in any of the paired normal gastric mucosa (Figure 1). A significant association was seen between IL-1α and COX-2 mRNA expression. IL-1α mRNA was expressed in 94% of COX-2-positive tumors, while it was expressed in only 25% of COX-2-negative tumors ($p < 0.0001$, Table I).

Figure 2. Effects of IL-1α on IL-1α (A) and COX-2 mRNA (B) expression in gastric cancer cell lines. MKN28 and MKN45 cells were treated with human recombinant IL-1α (0, 1 and 10 units/ml). IL-1α and COX-2 mRNA were detected by RT-PCR, and the ratio to GAPDH was plotted in the graph. The levels of IL-1α and COX-2 mRNA increased in an almost dose-dependent manner in both cell lines. RT-PCR was repeated three times and the representative results are shown in the figure.

Figure 3. Effects of IL-1α on cell growth in MKN28 (A) and in MKN45 (B) cells. Cell growth was shown as the OD value (n=4). A significant increase of the cancer cell growth was observed in both cell lines treated with human recombinant IL-1α (10 units/ml). *$p < 0.01$, at 48 h and 72 h of treatment.

Effect of IL-1α on expression of IL-1α and COX-2 mRNA in gastric cancer cells. In MKN28 and MKN45, the cells treated with recombinant human IL-1α showed an increase in both IL-1α and COX-2 mRNA levels (Figure 2A, 2B).

Effect of IL-1α on proliferation in gastric cancer cells. Exogenous IL-1α (10 units/ml) stimulated cell proliferation in both cell lines (Figure 3A, 3B). The OD value was significantly higher in the cells treated with IL-1α than in the cells without such treatment (control) at 48 h and 72 h in both cell lines ($p < 0.01$).

Both cell lines cultured in the medium (5% FBS) containing recombinant human IL-1α (10 units/ml) were then
treated with anti-IL-1α antibody, IL-1ra, or anti-COX-2 antibody and cell proliferation was assayed after 72 h of treatment. All three reagents inhibited cell growth stimulated by exogenous IL-1α in both cell lines (Figure 4A, 4B).

Discussion

In this study, IL-1α mRNA expression was up-regulated in 60% of human gastric cancer specimens and was related to the depth of tumor invasion. The in vitro study showed that exogenous IL-1α worked to produce IL-1α mRNA itself in gastric cancer cells. In addition, exogenous IL-1α was found to stimulate cell proliferation, while such growth stimulation was suppressed by inhibiting IL-1α activity using anti-IL-1α antibody or IL-1ra. IL-1α, therefore, might contribute to tumor progression in gastric cancer by stimulating cell proliferation. Our previous immunohistochemical analysis allowed us to localize IL-1α mainly within cancer cells (30). These results indicate that IL-1α secreted by cancer cells may act as an autocrine growth factor for gastric cancer. In addition, IL-1α has been shown to be produced by stromal cells, such as macrophages, fibroblasts and vascular epithelial cells (19). IL-1α secreted by stromal cells may also stimulate cancer cell growth in a paracrine manner.

Our RT-PCR analysis showed marked expression of IL-1α mRNA in most gastric cancer tissues showing COX-2 expression. The expression pattern of IL-1α mRNA was the same as that of COX-2, in that the positive bands were found only in cancer tissues, but not in any normal gastric mucosa specimens (14). Our in vitro study showed exogenous IL-1α to increase the levels of COX-2 mRNA in gastric cancer cell lines. In addition, IL-1α-stimulated cell growth was suppressed by inhibiting the COX-2 enzyme using the anti-COX-2 antibody. These findings demonstrated a critical link between IL-1α and COX-2 in human gastric cancer.

There is evidence that specific COX-2 inhibitors suppress cell growth and induce apoptosis in several tumor cell types (31-35), including gastric cancer cells (29), suggesting that the COX-2 enzyme itself may promote cancer progression by regulating proliferation and apoptosis (36). COX-2 was also found to contribute to the development and metastasis of human cancers via other mechanisms. COX-2-expressing cancer cells may increase in infiltrative potential due to the activation of metalloproteinase (37). PGs produced by COX-2 may facilitate tumor progression by acting as growth factors, as immunosuppressors and as angiogenic agents (16, 38-41). Our findings indicate that IL-1α is a growth factor which up-regulates the expression of the COX-2 enzyme in human gastric cancer. Thus, there is a strong possibility that IL-1α produced by cancer tissues may enhance COX-2 expression, and that the IL-1α-COX-2 pathway may facilitate the progression of gastric cancer tumors.

In conclusion, our data demonstrated that IL-1α and COX-2 mRNA were frequently co-expressed in human gastric cancer tissues, and suggested that the IL-1α-COX-2 pathway might play an important role in the progression of gastric cancer tumors. The IL-1α-COX-2 pathway may, therefore, provide an attractive target in therapy for human gastric cancers.

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References


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