Transcriptional Silencing of Cyclooxygenase-2 by Hyper-methylation of the 5 Cpg Island in Human Gastric Carcinoma Cells

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Background It has been well established that overexpression of Cyclooxygenase-2 (Cox-2) in epithelial cells inhibits apoptosis and increases the invasiveness of malignant cells, favoring tumorigenesis and metastasis. However, the molecular mechanism that regulates Cox-2 expression has not been well defined in gastric carcinoma.

Methods & Results We examined whether the Cox-2 expression could be regulated by hyper-methylation of the Cox-2 CpG island (spanning from 590 to +186 with respect to the transcription initiation site) in human gastric carcinoma cell lines. By Southern analysis, we found that 3 gastric cells (SNU-601, -620, -719) without Cox-2 expression demonstrated hyper-methylation at the Cox-2 CpG island. Detailed methylation pattern using bisulfite sequencing analysis revealed that all CpG sites were completely methylated in SNU-601. Treatment with demethylating agents effectively reactivated the expression of Cox-2 and restored IL-1 sensitivity in the previously resistant SNU-601. By transient transfection experiments, we demonstrate that constitutively active Cox-2 promoter activities were exhibited even without an exogenous stimulation in SNU-601. Furthermore, when the motif of NF-IL6 or CRE, or both, was subjected to point mutation, the constitutive luciferase activity was markedly reduced. In addition, Cox-2 promoter activity was completely blocked by in vitro methylation of all CpG sites in the Cox-2 promoter region with SsII (CpG) methylase in SNU-601.

Conclusion these results indicate that transcriptional repression of Cox-2 is caused by hyper-methylation of the Cox-2 CpG island in gastric carcinoma cell lines.