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 *Sss*I (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.