A QUANTITATIVE ANALYSIS OF CpG METHYLATION OF THE TRANSCRIPTION FACTOR 4 GENE IN GASTRIC CARCINOMA USING PYROSEQUENCING

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Background: Epigenetic silencing of tumor-related genes by CpG island methylation is an important mechanism for the development of many tumors, including gastric carcinoma. Deregulation of transcription factor 4 (TCF4) by promoter methylation was recently shown to play a key role in gastric carcinogenesis. As promoter methylation is a principal mechanism of epigenetically down-regulating genes, the status and level of CpG methylation in the promoter region of the TCF4 gene were analyzed in early and advanced gastric carcinomas (GC), compared with normal gastric tissues.

Materials and Methods: The extent of methylation in the TCF4 gene promoter was assessed, qualitatively and quantitatively, using methylation-specific PCR (MSP) and pyrosequencing (PS) in 60 early gastric carcinoma (EGC) and 60 advanced gastric carcinoma (AGC) samples collected during gastrectomy, and in 40 normal gastric mucosa samples collected from patients with benign gastric pathology. The gene product was analyzed by immunohistochemistry, and the relationship between the methylation profile of the promoter and clinicopathological variables was examined.

Results: A CpG island in the TCF-4 gene promoter was methylated in 81 (67.5%) of the 120 primary gastric carcinomas, based on MSP. The PS analysis of GCs revealed a higher frequency of TCF4 methylation (75.8%; 91/120). The methylation frequency for the TCF4 gene by both the MSP and PS techniques was significantly higher in advanced (75.0% and 91.7%, respectively) compared with early (60.0% and 60.0%, respectively, $p < 0.05$) GCs. There was a significant difference in TCF4 methylation between GCs and normal gastric tissues (67.5% vs. 40.0%, respectively, by MSP and 75.8% vs. 30.0%, respectively, by PS; $p < 0.05$). In the 120 GC samples analyzed, the results of the two methods were concordant in 94 (78.3%) and discordant in 26 (21.6%, $p < 0.001$) cases. Methylation of the TCF4 promoter as determined by both methods was closely correlated with reduced or absent TCF4 protein expression ($p < 0.001$). There was a significant correlation between TCF4 methylation status by PS and tumor size ($p = 0.004$), Lauren classification ($p = 0.043$), depth of invasion ($p < 0.001$), and nodal metastasis ($p = 0.021$).

Conclusions: These results suggest that TCF4 methylation occurs frequently in the early stage of gastric carcinoma progression and is significantly correlated with the down-regulation of TCF4 expression. Inactivation of the TCF4 gene by promoter methylation may play a role in the early stage of gastric carcinogenesis and may be associated with better prognosis. Furthermore, standard PCR followed by PS may provide a more specific and quantitative diagnostic alternative to MSP, because it produces a more comprehensive picture of the distribution of DNA methylation throughout the promoter regions of specific genes, which may be of benefit in oncology research.