Methylation pattern of CDH13 gene in digestive tract cancers

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Recently, the loss of CDH13 (T-cadherin, H-cadherin) gene expression accompanied by CDH13 promoter methylation was identified in colon cancers. We examined CDH13 methylation in oesophageal and gastric carcinomas. Five of 37 oesophageal cancers (14%) and 23 of 66 gastric cancers (35%) demonstrated abnormal methylation of the CDH13 promoter. Abnormal methylation was frequently found in gastric cancers of patients at all clinical stages just as in E-cadherin, another of the cadherin family, suggesting that these cancers could be methylated at an early stage. These results suggested that CDH13 might play a variety of roles depending on the tissue type.

Keywords: CDH13; oesophageal cancer; gastric cancer

Advances in molecular genetics have established that several genetic changes, such as the activation of the K-ras oncogene and inactivation of the p53 tumour suppressor gene, are involved in the pathogenesis of colorectal and other cancers (Bos et al, 1987; Vogelstein et al, 1988; Baker et al, 1989). Recently, a growing number of cancer genes are being recognised that harbour hypermethylation of a normally unmethylated promoter CpG islands (Jones and Laird, 1999; Baylin and Herman, 2000). This epigenetic change results in no expression of tumour suppressor gene and plays a key role in an epigenetically mediated loss-of-gene function that is as critical for tumorigenesis as mutations in coding regions.

In fact, it has been confirmed that hypermethylation of a normally unmethylated promoter CpG islands in the promoter region of p16 (Jones and Laird, 1999; Baylin and Herman, 2000). This epigenetic change results in no expression of tumour suppressor gene and plays a key role in an epigenetically mediated loss-of-gene function that is as critical for tumorigenesis as mutations in coding regions. However, it is not clear whether the epigenetic changes are specific to tumour suppressor genes or whether they are more generally applicable to other genes involved in tumourigenesis.

As a result, we have explored the involvement of DNA methylation in the pathogenesis of digestive tract cancers and have identified that CDH13 was also inactivated by methylation in other digestive tract cancers. In this study, we first examined the methylation status and gene expression of CDH13 in digestive tract cancer cell lines using methylation-specific PCR (MSP) and reverse transcription–PCR (RT–PCR), respectively. We then examined CDH13 methylation in oesophageal and gastric carcinomas. The results obtained were then compared to the clinicopathological features.

MATERIALS AND METHODS

Sample collection and DNA preparation

Three colorectal cancer cell lines (SW1083, SW1222, and SW1417), one gastric cancer cell line (MKN1), and one oesophageal squamous cancer cell line (TE1) were kindly provided by the Memorial Sloan-Kettering Cancer Center (New York, NY, USA) or purchased from the American Type Culture Collection (Manassas, VA, USA). Two gastric (NUGC3 and NUGC4) and two oesophageal squamous cancer cell lines (NUEC1 and NUEC2) were established in our laboratory. Primary tumours and corresponding normal tissues were obtained from the Nagoa University Hospital from 37 oesophageal squamous cell cancer and 66 gastric cancer patients who had been diagnosed histologically. These samples were obtained during surgery. All cancer specimens contained more than 70% neoplastic cells. This was confirmed using paraffin-embedded tissues stained by haematoxylin and eosin. Oral or written informed consent, as indicated by the institutional review board, was obtained from all patients. All tissues were quickly frozen in liquid nitrogen and stored at −80°C until analysis. Cell line and tumour DNA were prepared as described previously (Hibi et al, 1998).

Bisulphite modification and MSP

DNA from tumour and normal tissue specimens was subjected to bisulphite treatment as described previously (Hibi et al, 1998). The modified DNA was used as a template for MSP. Primer sequences...
of CDH13 for amplification were described previously (Sato et al., 1998). The primers for the unmethylated reaction were: CDH13UMS (sense), 5'-TTGTTGGGTTGTGTTTGT, and CDH13UMAS (antisense), 5'-AACATTTCATCATCACACA, which amplify a 242 bp product. The primers for the methylated reaction were: CDH13MS (sense), 5'-TCGGGCGGTTGTTTTCGC, and CDH13MAS (antisense), 5'-GAGTGGTCATCCACGCG, which amplify a 243 bp product. The PCR amplification of modified DNA samples consisted of one cycle of 95°C for 5 min, 33 cycles of 95°C for 30 s, 60°C for 1 min, and 72°C for 1 min for the unmethylated reaction or 29 cycles of 95°C for 30 s, 70°C for 1 min, and 72°C for 1 min for the methylated reaction, and one cycle of 72°C for 5 min. DNAs from TE1 (oesophageal squamous cell cancer cell line) and SW1417 (colon cancer cell line) were used as positive controls of CDH13 amplification for unmethylated and methylated alleles, respectively. The methylation status of SW1417 cells has already been examined previously (Toyooka et al., 2002). Controls without DNA were performed for each set of PCR. In all, 10μl of each PCR product was directly loaded onto nondenaturing 6% polyacrylamide gels, stained with ethidium bromide, and visualised under UV illumination. Each MSP was repeated at least three times.

Reverse transcription–PCR (RT–PCR)

First-strand cDNA was generated from RNA as described previously (Hibi et al., 1991). The PCR amplification consisted of 30 cycles (95°C for 30 s, 55°C for 1 min, and 72°C for 1 min) after the initial denaturation step (95°C for 2 min). The primers used were: CDH13-S (sense), 5'-TCGACGACAAAAAGTGTTTCATAT, and CDH13-AS (antisense), 5'-GTGGTAGGGACACAGAGT. Primer sequences were described previously (Sato et al., 1998). The predicted size of PCR product was 208 bp. The housekeeping gene, β-actin, was used as an internal control to confirm the success of the RT reaction.

Statistical analysis

The χ² (Fisher's exact) test and Student's t-test were used to examine the association between CDH13 promoter methylation and clinicopathological features.

RESULTS

We first examined the methylation status of CDH13 in digestive tract cancer cell lines using MSP. DNA from all three colorectal cancer cell lines (SW1083, SW1222, and SW1417), two of three gastric cancer cell lines (MKN1, NUGC3, and NUGC4), and none of three oesophageal cancer cell lines (TE1, NUEC1, and NUEC2) exhibited abnormal promoter methylation of CDH13 gene (Figure 1). To confirm the status of CDH13 gene according to the methylation pattern, we next examined CDH13 expression in these cell lines using RT–PCR. Three colon and two gastric cancer cell lines that demonstrated only methylation of the CDH13 promoter lacked CDH13 gene expression, while CDH13 was expressed in all other cell lines with unmethylation of the CDH13 promoter including one gastric and three oesophageal cancer cell lines (Figure 1).

Subsequently, we examined whether aberrant methylation could be detected in primary oesophageal and gastric cancers. Five of 37 oesophageal cancers (14%) and 23 of 66 gastric cancers (35%) demonstrated abnormal methylation of the CDH13 promoter. In our previous study, CDH13 methylation was detected in 27 of 84 primary colorectal cancers (32%) (data not shown). Representative results of MSP analyses of CDH13 promoter are shown in Figure 2. As a control, we screened for CDH13 methylation in the corresponding normal epithelial DNA of 37 oesophageal and 66 gastric cancer patients. No methylation was found in the normal DNA of this control group. As Figure 2 showed, all cases exhibited unmethylation to a greater or lesser extent. Therefore, it might be possible that the CDH13 gene expression has not been inhibited completely in these cancers. It might also be possible that DNA derived from inflammatory and interstitial cells among cancer cells exhibited unmethylation because it is impossible to exclude these cells completely from cancer cells obtained for this study.

After methylation analysis of all samples, clinicopathological data were correlated with these results. Sex, age, extent of tumour, clinical stage, lymph node metastasis, histology, and prognosis were not significantly correlated with representations of abnormal methylation in oesophageal or gastric cancers (Tables 1 and 2). Compared with CDH13-unmethylated cancers, CDH13-methylated cancers showed a trend towards preferentially invasive (P = 0.140) and short time alive (P = 0.167) in oesophageal cancers. On the
other hand, abnormal methylation was found in gastric cancers of patients at all clinical stages, suggesting that these cancers could be methylated at an early stage.

**DISCUSSION**

Several tumour suppressor genes contain CpG islands in their promoters, prompting many studies investigating the role of methylation in silencing these genes. Many tumour suppressor genes show evidence of methylation silencing, providing a new potential pathway for the deactivation of tumour suppressor genes (Jones and Laird, 1999).

**CDH13**

one among the cadherin family, would be a cell surface glycoprotein responsible for cell adhesion. Recently, it was reported that the promoter of E-cadherin, another of the cadherin family, frequently underwent hypermethylation in human gastric cancers (Tamura et al., 2000). Therefore, it is conceivable that **CDH13** was also inactivated in gastric cancers by promoter methylation. In this study, **CDH13** gene was methylated frequently in gastric cancers, suggesting that the inactivation of this gene plays an important role in this cancer while it does not do so in oesophageal squamous cell cancers. Moreover, abnormal methylation was found in gastric cancers of patients at all clinical stages, suggesting that these cancers could be methylated at an early stage. These results suggested that **CDH13** might play various roles depending on the tissue types along the digestive tract.

As previously described, the methylation of **CDH13** gene would not be complete, suggesting that the **CDH13** gene expression has not been completely inhibited in primary cancers. Zheng et al. (2000) reported previously that a partial methylation pattern was
associated with relatively low levels of p14ARF in colorectal cancer cell lines. p14ARF mRNA was expressed at extremely low levels in fully methylated cell lines, and p14ARF expression in the partially methylated LoVo cell line was intermediate. Moreover, partial methylation of p14ARF was the most common pattern observed in primary colorectal cancers. Taken together, these findings suggest that the level of CDH13 gene expression might also be controlled by methylation in primary cancers.

Our results suggested that the aberrant methylation of CDH13 gene has been shown frequently in oesophageal and gastric cancers. In addition, abnormal methylation was found in gastric cancers of patients at all clinical stages, suggesting that these cancers could be methylated at an early stage. Therefore, CDH13 methylation could be used as a tumour marker in clinical samples such as serum and stool for the early detection of digestive tract cancers (Hibi et al., 2001; Kanyama et al., 2003).

Recent studies have shown that it is possible to reverse epigenetic changes and restore gene function to a cell. Treatment with DNA methylation inhibitors can restore the activities of CDH13 gene and decrease the growth rate of cancer cells. The administration of drugs such as cytosine analogues might soon be able to restore the function of these tumour suppressor genes and slow the rate of cancer progression.

ACKNOWLEDGEMENTS

We thank M Taguchi for her technical assistance.

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