Loss of heterozygosity and mRNA expression at deleted in colorectal cancer gene locus in gastric cancer

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INTRODUCTION

Tumor suppressor genes play an important role in regulating normal cellular proliferation [1-3]. Conversely, inactivation of tumor suppressor genes at both alleles may allow a cell to lose normal growth controls and acquire a malignant phenotype. This inactivation may occur through a variety of mechanisms including deletion, rearrangement, point mutation, gene conversion, and binding of suppressor gene products with viral or cellular inactivating proteins [4-6]. The deleted in colorectal cancer (DCC) gene was first cloned based on frequent deletions affecting the 18q21 region in colon cancer [7]. Subsequently, loss of heterozygosity (LOH) or loss of expression (LOE) of DCC has been reported in several other tumor types, including breast [8,9], pancreatic [10], prostate [11] and testicular [12] carcinomas, glioblastomas [13] and hematological malignancies [14].

In a study of human gastric cancer, chromosome 18q was frequently affected by the loss of heterozygosity detectable in more than 60% of cases [15,16]. However, there have been no studies reported on LOE of DCC gene in gastric cancer. In order to investigate the effects of the DCC gene abnormality on the development and progression of gastric cancer, LOH and LOE of DCC gene were examined using a PCR based detection method.

MATERIALS AND METHODS

Tissue specimens

Tumor and corresponding noncancerous tissues were obtained from 51 patients who underwent surgical resection for gastric carcinoma between January 1993 and October 1996 at the Southwest Hospital. None of the patients had received any radiotherapy or chemotherapy preoperatively. Each pair of tumor and corresponding non-tumor tissues was stored at -80 °C immediately after the resection for experimental use. A 6 μm section was cut from each tissue and stained with hemotoxylin/eosin for pathological diagnosis. After diagnostic confirmation, a visual assessment was made of the approximate proportion of tumor cells vs normal cells in the tumor. Only the specimens in which tumor cells represented ≥ 60% of the tumor tissue were accepted for LOH and LOE analysis.

Total RNA isolation and DNA extraction

Total RNA was prepared from tumor and noncancerous tissues using the acid guanidinium thiocyanate method [17] and high molecular weight DNA was extracted using proteinase K digestion and phenol chloroform isomyl alcohol extraction as previously described [18].

RT-PCR assay of DCC gene expression

RT-PCR was performed as described previously with some modifications [19]. DCC complementary DNA was amplified at 94 °C for 40 s; 49 °C for 40 s, and 72 °C for 1 min in a Perkin Elmer Thermocycler 2400 for 35 cycles. DCC primers were located on exons O and P, amplifying a 233 base pair fragment.
Table 1  Relationship of loss of heterozygosity (LOH) and mRNA expression (LOE) of deleted in colorectal cancer (DCC) with clinicopathological parameters

| Clinicopathologic parameters | LOH/informative (%) | LOE/No. examined (%) |
|------------------------------|----------------------|-----------------------|
| Differentiation              |                      |                       |
| Well/moderate                | 4/12 (33.3)          | 1/6 (16.7)            |
| Poor                         | 12/28 (42.9)         | 5/14 (35.7)           |
| Mucinous carcinoma           | 2/11 (18.2)          | 4/20 (20.0)           |
| Tumor size                   |                      |                       |
| < 5 cm                       | 4/20 (20.0)          | 4/12 (33.3)           |
| > 5 cm                       | 14/33 (42.4)         | 5/14 (35.7)           |
| Sensory invasion             |                      |                       |
| Absent                       | 4/18 (22.2)          | 3/12 (25.0)           |
| Present                      | 12/33 (36.4)         | 5/14 (35.7)           |
| Lymph node metastasis        |                      |                       |
| Absent                       | 5/24 (20.8)          | 2/13 (15.4)           |
| Present                      | 13/27 (48.1)         | 6/13 (46.2)           |
| Clinical staging             |                      |                       |
| Stages I-II                  | 3/21 (14.3)          | 2/11 (18.2)           |
| Stages III-IV                | 15/30 (50.0)         | 6/15 (40.0)           |

*P < 0.05 as compared with that of stage I and II.

Table 2  Relationship between loss of heterozygosity (LOH) and mRNA expression (LOE) of deleted in colorectal cancer (DCC) gene

| Groups | LOH | LOE | No. of cases (%) |
|--------|-----|-----|------------------|
| 1      | +   | *   | 4 (15.4)         |
| 2      | -   | -   | 14 (53.8)        |
| 3      | 3   | 4   | 4 (15.4)         |
| 4      | +   | -   | 4 (15.4)         |

+ Positive; and - Negative.

RESULTS

LOH of the DCC gene was determined by PCR-LOH in 51 specimens of gastric cancer. In order to raise the assay sensitivity, three different sites, i.e., M2, M3 and VNTR were used in this study. LOH of DCC was observed in 9 of 47 (19.0%) at M2, 7 of 50 (14.0%) at M3 and 3 of 26 (11.5%) at VNTR sites, respectively. If a positive allelic deletion of DCC was judged by LOH at one or any combination of these three sites, the incidence of LOH at the DCC locus was 35.3% (18/51). LOH was detected in 37.5% (6/16) of intestinal type of gastric cancer and 36.4% (12/33) of gastric type. Of the 51 cases of gastric cancer, 26 underwent the examination of expression of DCC mRNA, and LOE was observed in 30.8% (8/26) (Figure 4). The incidence of LOE was 44.4% (4/9) in the intestinal type and 23.5% (4/17) in gastric type. χ² test revealed no significant difference of the LOH and LOE between these two types of cancer (p > 0.05).

Correlation between LOH and LOE of DCC and clinicopathological data of gastric cancer are illustrated in Table 1. χ² test demonstrated that LOH of DCC was significantly higher in stages III and IV gastric cancer than that in stage I or II (P < 0.05).
LOH and LOE is shown in Table 2. The paired data were analyzed with χ² test and no significant correlation was found between LOH and LOE (p = 0.05).

DISCUSSION

The DCC gene is located on the human chromosome 18q11-12. It was reported that the inactivation of this gene is closely related to the pathogenesis of colorectal, esophageal and pancreatic carcinoma, and this gene is considered a susceptible gene in gastrointestinal tumours. Uchino et al.8,9 and Ranzani et al.10,11 reported that the rate of LOH of DCC in gastric cancer was 61% and 42.9%, respectively; in our study, this rate was 35.5%. Together, these results suggest that DCC gene takes part in the pathogenesis of gastrointestinal cancer through its LOH.

To our knowledge, this is the first report on the expression of mRNA of DCC gene in gastric cancer, which confirmed the LOE of DCC mRNA in the gastric cancers with RT-PCR. This suggests that the inactivation of DCC in gastric cancer occurs in various patterns, and further confirms that the DCC gene is the susceptible gene of gastric cancer, playing an important role in the pathogenesis of gastrointestinal cancer.

The histological progression associated with the intestinal type of gastric cancer has been well documented, with apparent evolution through a sequence of superficial gastric, intestinal metaplasia and dysplasia12,13. Lesions indicating an adenoma carcinoma sequence similar to that in the colorectum were also observed in the stomach14,15. In the present study we have found that the rate of LOE of DCC was as high as 44.4% in intestinal type of gastric cancer, which approached that of the rate seen in colorectal cancer15,16. However, the rate of LOE was rather low in gastric type of gastric cancer. Though there was no statistical difference between the two types of gastric cancer, similarities in genetic alterations between colorectal and intestinal type of carcinoma may reflect a carcinogenetic pathway common to colorectal carcinoma and gastric cancer. It was also found in our study that the rate of LOH of intestinal type of DCC was similar between the two types of gastric cancer. Whether the effects of LOH and LOE of DCC gene are different between the two types of gastric cancer needs further studies.

The DCC gene encodes a molecule which shares high homology with the neural cell adhesion molecule17,18. Cell adhesion molecules are cell surface receptors that play critical roles in a number of different processes, including embryogenesis, thrombosis, wound healing, cell homing, and immunoreactivity, as well as tumor progression and metastasis. The inactivation of DCC gene may result in malignant degeneration of the cells and aid in the invasion and metastasis of a tumor. Kato et al.19,20 found that the incidence of LOH at DCC locus in colorectal cancer was significantly greater for patients with liver metastasis than for patients with no liver metastasis. Lino et al.21 and Yanooshita et al.22 observed that the expression of DCC gene in mRNA was greatly reduced or not detectable in invasive colorectal carcinoma in comparison with carcinoma in adenoma and intramucosal carcinoma. They indicated that the inactivation of the DCC gene was associated with the progression of early stage carcinoma to advanced stage. Itoh et al.23 revealed that the expression level of DCC mRNA was lower in liver metastasis than in primary carcinoma. These findings imply that the inactivation of the DCC gene occurs in the late stage of colorectal carcinoma, and was of prognostic significance. There were similar conclusions in the study of esophageal and pancreatic carcinoma24,25. In our study, the rate of LOH of DCC rose along with the increase of tumor size and the depth of invasion and the metastasis to lymph nodes, and LOE of DCC frequently occurred in gastric cancer of stages II and IV with lymph node metastasis. Though these data did not find a statistical significance, they may suggest that DCC gene plays a definite role in the proliferation, invasion and metastasis of gastric cancer. The LOH rate of DCC in our study was significantly higher in the stages III and IV than that in stages I or II, which indicates that LOH of DCC occurs in the late stage and is related to the advances of the malignancy. Ranzani et al.10,11 had similar findings as ours. Thus, it is expected that LOH and LOE of DCC may be potentially used as a prognostic factor for gastric cancer. However, to accurately reveal the correlation of LOH and LOE of DCC with clinicopathologic factors and prognosis, a larger number of cases should be examined.

The interrelation of LOH and LOE of DCC gene was preliminarily studied and it was found that LOH does not seem to be necessary for LOE of DCC mRNA, which was similar to the finding of others26,27. There might be some other causes, such as alternations in sequences controlling transcriptional regulation, point mutation or insertions within the DCC gene, or alterations in other gene controlling DCC expression. Further studies are required to examine these possibilities.
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