Analysis of the *DPC4* Gene in Gastric Carcinoma

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Allelic loss on chromosome 18q is involved in the progression of gastric carcinoma. Recently, *DPC4* (deleted in pancreatic carcinoma, locus 4), a candidate tumor suppressor gene, has been localized at 18q21.1. This gene is inactivated in nearly one half of pancreatic carcinomas. We tested for *DPC4* gene mutations and allelic status at 18q21 in 30 primary gastric carcinomas and 5 gastric carcinoma cell lines. Polymerase chain reaction single-strand conformation polymorphism and sequencing analyses revealed no *DPC4* mutations in any of the primary tumors or cell lines. Homozygous deletion of *DPC4* was observed in only 1 (MKN-45) of the 5 (20%) cell lines. This suggests that the target gene for loss on 18q is not *DPC4*. The true tumor suppressor gene, encoded near *DPC4*, has yet to be identified.

Key words: *DPC4* — Chromosome 18q — Tumor suppressor gene — Gastric carcinoma

Loss of heterozygosity (LOH) on the long arm of chromosome 18 (18q) is frequent in gastric carcinoma.[1-3] The *DCC* (deleted in colorectal carcinomas) gene, which is located at 18q21,[4] has been postulated to be the major target of the loss on 18q in gastric carcinomas, though its mutational status in gastric carcinoma is unknown because of the length and complexity of the gene.[5] Recently, *DPC4* (deleted in pancreatic carcinoma, locus 4) has been identified at 18q21.1 by positional cloning.[6] The frequent inactivation of this gene, with homozygous deletion or mutations accompanied with LOH in pancreatic carcinomas has been reported, suggesting that the gene may be a tumor suppressor.[6] In the present study, we tested for *DPC4* gene mutations and allelic losses at 18q21 to determine the significance of this gene in gastric carcinogenesis.

Thirty primary gastric carcinomas (28 differentiated, 2 undifferentiated) obtained surgically from 30 patients, and 5 gastric carcinoma cell lines (MKN-1, MKN-7, MKN-45, MKN-74, and HGC-27) obtained from the Riken Gene Bank (Tsukuba Science City) were examined. Mutations in all 11 exons of *DPC4* were screened using polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis followed by direct sequencing. The PCR primers used for the analysis of *DPC4* have been described by Kim *et al.*[7] Allelic loss was examined using 6 polymorphic microsatellite markers, *D18S460, D18S474, D18S46, D18S363, DCC,* and *D18S69*, obtained from MapPairs (Research Genetics, Huntsville, AL), at 18q21. These markers were mapped

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by Hahn *et al.*,[6] Overhauser *et al.*[8] and Eppert *et al.*[9] The disparity of *D18S363* localization between Hahn *et al.* and Eppert *et al.*[6,9] has been noted. We ordered the marker according to the report of Hahn *et al.*[6] as a critical locus together with *D18S46* for allelic loss, because frequent homozygous deletion in pancreatic carcinomas has been reported.[10] Homozygous deletions in the cell lines were defined as the absence of PCR products of all 11 exons and microsatellite markers from individual DNA templates with comparative multiplex PCR. The microsatellite marker, *D18S474*, which was retained in all cell lines, was selected as an internal control. The procedures for PCR-SSCP, direct sequencing, and PCR-microsatellite assays have been described previously.[11,12]

Three of the 30 primary tumors exhibited mobility shift bands by SSCP (Fig. 1). DNA sequencing analysis of the bands with altered mobility revealed the same nucleotide change in intron 7 in 3 tumors (Fig. 2). Therefore, this nucleotide change is likely to be a polymorphism. A homozygous deletion of all 11 exons was observed in 1 (MKN-45) of the 5 (20%) cell lines. No structural abnormalities were found in the remaining 4 cell lines. The expression of full-length *DPC4* mRNA was detected by RT-PCR in 4 cell lines, while no detectable expression was observed in the MKN-45 cell line (data not shown), confirming that the *DPC4* gene is inactivated by homozygous deletion in MKN-45. LOH at any locus on 18q21 occurred in 10 of 30 (33%) primary tumors (Fig. 3). No homozygous deletions were found in any of the 5 cell lines at any microsatellite locus. A deletion map of the 18q region in the primary tumors is shown in Fig. 4.
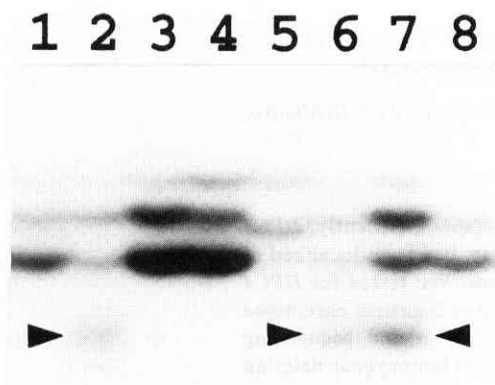
1 2 3 4 5 6 7 8

Fig. 1. SSCP analysis of exon 7 in *DPC4*. Lane 1, patient #135; lane 2, #140; lane 3, #143; lane 4, #145; lane 5, #150; lane 6, #151; lane 7, #153; and lane 8, #154. The bands with a mobility shift in lanes 2, 6, and 7 are indicated by arrowheads.

A

GTGGTGATTGAAACGCACAAACACAGG
140   150   160

A

↗C to T

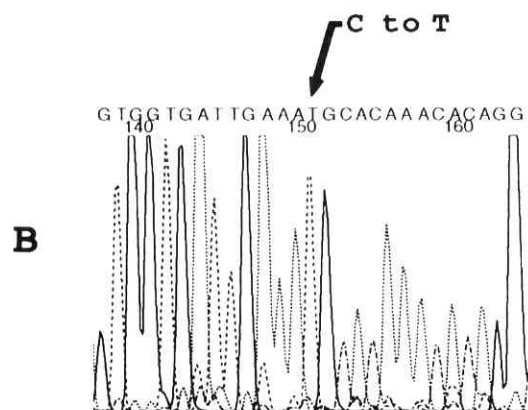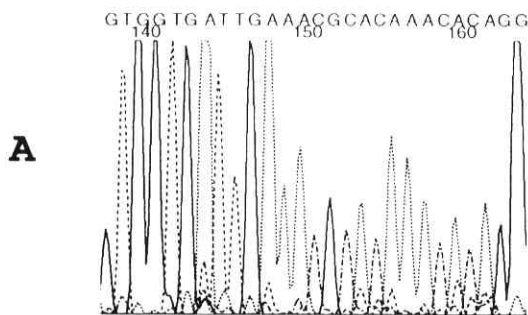GTGGTGATTGAAATGCACAAACACAGG
140   150   160

B

Fig. 2. Sequencing histogram of *DPC4*. A, Wild-type sequence of intron 7. B, Sequence of the mobility shift band from patient #140. A single base nucleotide change (C to T) is shown.

A

135   134   150
T   N   T   N   T   N

D18S474

D18S46

DCC

B

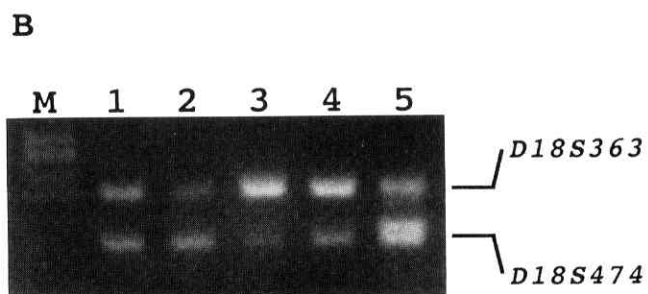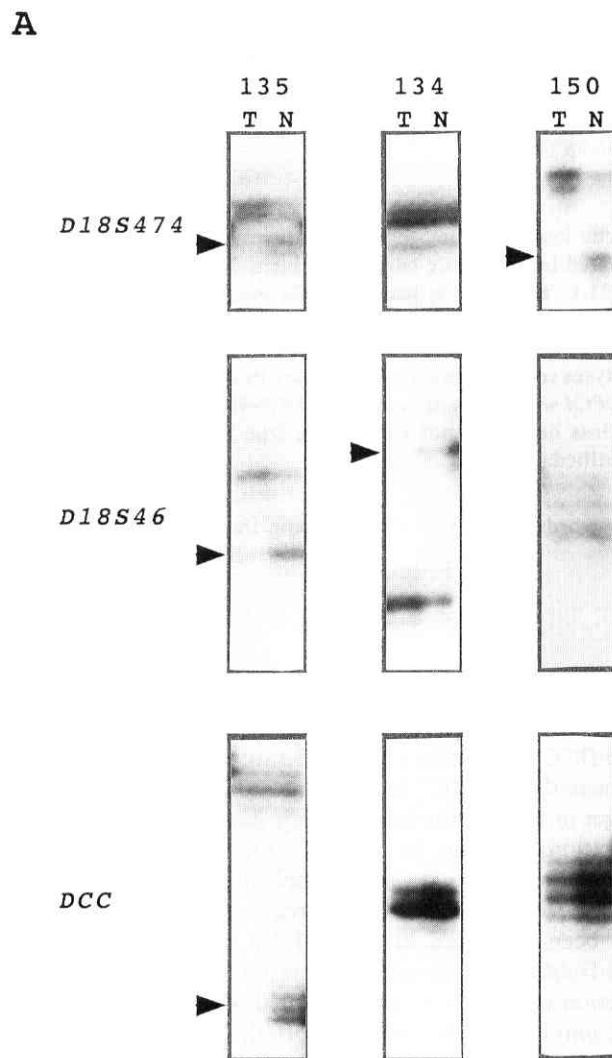M   1   2   3   4   5

D18S363

D18S474

Fig. 3. Representative illustrations of microsatellite analyses in primary tumors (A) and cell lines (B). A, Loss of heterozygosity (LOH) is indicated by arrowheads. T, tumor DNA; N, normal DNA. B, Multiplex PCR of the cell lines. No homozygous deletions were present. Lane 1, MKN-1; lane 2, MKN-7; lane 3, MKN-45; lane 4, MKN-74; lane 5, HGC-27; M, size marker.
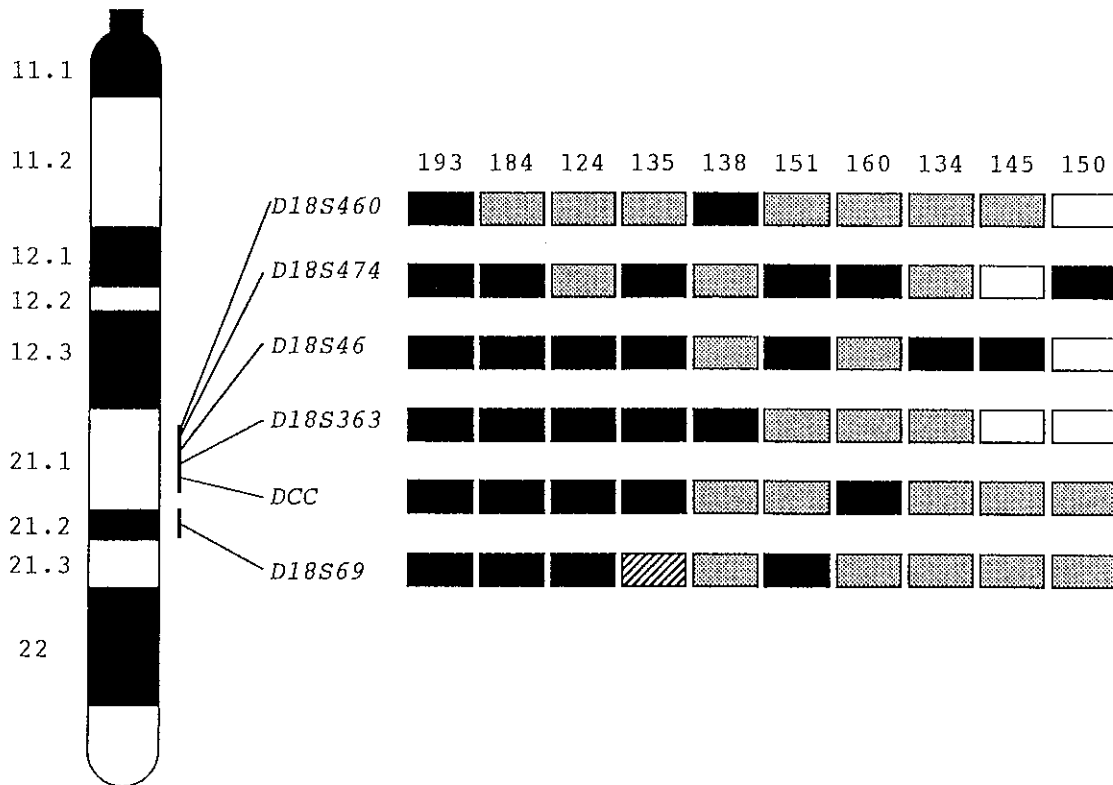
Fig. 4. Deletion map of 18q in primary gastric carcinomas (case numbers 193, 184, 124, 135, 138, 151, 160, 134, 145, and 150). Black symbols indicate LOH. Open symbols indicate those that retained heterozygosity. Gray symbols indicate homozygosity. Dashed symbols indicate replication error.

Homozygous deletion at 18q21.1, in which the *DPC4* gene is located, is frequent in pancreatic carcinoma.[10] Several investigators have analyzed various tumor types for alterations in the *DPC4* gene.[6, 7, 13, 14] They found that the inactivation of *DPC4* is rare in carcinomas other than pancreatic carcinoma, such as carcinomas of the head and neck, breast, lung, ovary, prostate, and esophagus, although LOH at 18q21.1 was observed in over 30% of these tumors.[6, 7, 13, 14] Frequent LOH on 18q in gastric carcinoma has been reported previously, and it may play a crucial role in gastric carcinogenesis.[1–3] In our previous allelotype analysis of gastric carcinoma, we found frequent LOH (36%) at D18S69, located about 3 cM away from *DPC4*, and telomeric to *DCC*.[3] Thus, *DCC* was postulated to be the target of LOH on 18q21 in gastric carcinoma. In the present study, we have demonstrated that LOH at *D18S474* and *D18S46* spanning *DPC4* was more frequent than at *DCC* and *D18S69* (Table I). From these results, it appeared likely that the target of loss on 18q is *DPC4* rather than *DCC*. However, no structural alterations in *DPC4* were detected in gastric carcinomas.

Table I. Loss of Heterozygosity (LOH) on 18q in Primary Gastric Carcinomas

Locus symbol	Location	Frequency of informative cases	Frequency of LOH
D18S460	18q21.1	23% (7/30)	29% (2/7)
D18S474	18q21.1	50% (15/30)	40% (6/15)
D18S46	18q21.1	60% (18/30)	39% (7/18)
D18S363	18q21.1	63% (19/30)	32% (6/19)
DCC	18q21.1	67% (20/30)	25% (5/20)
D18S69	18q21.2	47% (14/30)	29% (4/14)

Our present data indicate that the inactivation of *DPC4* by a conventional two-hit mechanism does not contribute to gastric carcinogenesis. One (MKN-45) of the five cell lines exhibited a homozygous deletion of *DPC4* without the loss of any microsatellite markers at 18q21. The remaining four cell lines did not show homozygous deletion of *DPC4*, or any microsatellite locus examined. It

has been reported that the markers *D18S46* and *D18S363* were homozygously deleted in 4 of 31 pancreatic carcinoma xenografts.[10] These markers did not involve the *DCC* region, and are located centromeric to *DCC*.[10]

It has been suggested that 18q21 is a locus for multiple tumor suppressor genes.[9] DPC4 has sequence similarity to *Drosophila melanogaster* mathers against dpp (MAD) protein, which is implicated in a transforming growth factor β (TGF-β)-like signaling pathway,[6] and it has been considered to be one of the MAD-related genes. Recently, Eppert *et al.*[9] have identified another MAD-related protein, MADR2, and mapped the gene to 18q21.1, close to *DPC4*. MADR2 protein is an essential component in the TGF-β signaling pathway, and it is regarded as a candidate tumor suppressor because *MADR2* is inactivated in sporadic colorectal carcinomas by a conventional two-hit mechanism.[9] Therefore, at least two candidate tumor suppressor genes are encoded at 18q21.1. This may be similar to the cases of *APC* (adenomatous polyposis coli) and *MCC* (mutated in colon cancer) on 5q21.[15] We demonstrated that LOH at *D18S460*, which was mapped very close to the *MADR2*

gene, was less frequent than that at the *DPC4* locus. In addition, mutational analyses with reverse transcriptase-PCR-SSCP[9] have revealed no mobility shifts in 5 gastric carcinoma cell lines (data not shown). Hence, it is unlikely that *DPC4* or *MADR2* contributes to gastric carcinogenesis. Although the relationship between *DPC4* and *MADR2* remains to be elucidated, Eppert *et al.*[9] have suggested that MADs may represent a new class of tumor suppressor genes in humans.

In the present study we were unable to detect any mutations in *DPC4* in primary gastric carcinomas or gastric carcinoma cell lines, despite the frequent LOH in primary tumors and a homozygous deletion in the MKN-45 cell line. This suggests that one or more other tumor suppressor genes are encoded at 18q21.1.

REFERENCES

1) Uchino, S., Tsuda, H., Noguchi, M., Yokota, J., Terada, M., Saito, T., Kobayashi, M., Sugimura, T. and Hirohashi, S. Frequent loss of heterozygosity at the *DCC* locus in gastric cancer. *Cancer Res.*, **52**, 3099–3102 (1992).

2) Maesawa, C., Tamura, G., Suzuki, Y., Ogasawara, S., Sakata, K., Kashiwaba, M. and Satodate, R. The sequential accumulation of genetic alterations characteristic of the colorectal adenoma-carcinoma sequence does not occur between gastric adenoma and adenocarcinoma. *J. Pathol.*, **176**, 249–258 (1995).

3) Tamura, G., Sakata, K., Nishizuka, S., Maesawa, C., Suzuki, Y., Terashima, M., Eda, Y. and Satodate, R. Allelotype of adenoma and differentiated adenocarcinoma of the stomach. *J. Pathol.*, **180**, 371–377 (1996).

4) Fearon, E. R., Cho, K. R., Nigro, J. M., Kern, S. E., Simons, J. W., Ruppert, J. M., Hamilton, S. R., Preisinger, A. C., Thomas, G., Kinzler, K. W. and Vogelstein, B. Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science*, **247**, 49–56 (1990).

5) Cho, K. R., Oliner, J. D., Simons, J. W., Hedrick, L., Fearon, E. R., Preisinger, A. C., Hedge, P., Silverman, G. A. and Vogelstein, B. The DCC gene: structural analysis and mutations in colorectal carcinomas. *Genomics,* **19**, 525–531 (1994).

6) Hahn, S. A., Schutte, M., Hoque, A. T. M. S., Moskaluk, C. A., da Costa, L. T., Rozenblum, E., Weinstein, C. L., Fischer, A., Yeo, C. J., Hruban, R. H. and Kern, S. E. *DPC4*, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science,* **271**, 350–353 (1996).

7) Kim, S. K., Fan, Y., Papadimitrakopoulou, V., Clayman, G., Hittelman, W. N., Hong, W. K., Lotan, R. and Mao, L. *DPC4*, a candidate tumor suppressor gene, is altered infrequently in head and neck squamous cell carcinoma. *Cancer Res.*, **56**, 2519–2521 (1996).

8) Overhauser, J., Silverman, G. A., Gerken, S. and van Kessel, A. G. Report of the third international workshop on human chromosome 18 mapping 1995. *Cytogenet. Cell Genet.*, **71**, 105–119 (1995).

9) Eppert, K., Scherer, S. W., Ozcelik, H., Pirone, R., Hoodless, P., Kim, H., Tsui, L.-C., Bapat, B., Gallinger, S., Andrulis, I. L., Thomsen, G. H., Wrana, J. L. and Attisano, L. MADR2 maps to 18q21 and encodes a TGFβ-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. *Cell,* **86**, 543–552 (1996).

10) Hahn, S. A., Hoque, A. T. M. S., Moskaluk, C. A., da Costa, L. T., Schutte, M., Rozenblum, E., Seymour, A. B., Weinstein, C. L., Yeo, C. J., Hruban, R. H. and Kern, S. E. Homozygous deletion map at 18q21.1 in pancreatic cancer. *Cancer Res.,* **56**, 490–494 (1996).

11) Tamura, G., Ogasawara, S., Nishizuka, S., Sakata, K., Maesawa, C., Suzuki, Y., Terashima, M., Saito, K. and Satodate, R. Two distinct regions of deletion on the long arm of chromosome 5 in differentiated adenocarcinomas of the stomach. *Cancer Res.,* **56**, 612–615 (1996).

12) Maesawa, C., Tamura, G., Nishizuka, S., Ogasawara, S., Ishida, K., Terashima, M., Sakata, K., Sato, N., Saito, K. and Satodate, R. Inactivation of the *CDKN2* gene by homozygous deletion and *de novo* methylation is associated

with advanced stage esophageal squamous cell carcinoma. *Cancer Res.,* **56,** 3875–3878 (1996).

13) Schutte, M., Hruban, R. H., Hedrick, L., Cho, K. R., Nadasdy, G. M., Weinstein, C. L., Bova, G. S., Isaacs, W. B., Cairns, P., Nawroz, H., Sidransky, D., Casero, Jr. R. A., Meltzer, P. S., Hahn, S. A. and Kern, S. E. *DPC4* gene in various tumor types. *Cancer Res.,* **56,** 2527–2530 (1996).

14) Barrett, M. T., Schutte, M., Kern, S. E. and Reid, B. J. Allelic loss and mutational analysis of *DPC4* gene in esoph-

ageal adenocarcinoma. *Cancer Res.,* **56,** 4351–4353 (1996).

15) Kinzler, K. W., Nilbert, M. C., Su, L.-K., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hedge, P., McKechnie, D., Finniear, R., Markham, A., Groffen, J., Boguski, M. S., Altschul, S. F., Horii, A., Ando, H., Miyoshi, Y., Miki, Y., Nishisho, I. and Nakamura, Y. Identification of FAP locus genes from chromosome 5q21. *Science,* **253,** 661–665 (1991).