RAPID COMMUNICATION Jpn. J. Cancer Res. 88, 701–704, August 1997

Infrequent Mutation of the H-Cadherin Gene on Chromosome 16q24 in Human Breast Cancers

Yoshio Miki,[1] Toyomasa Katagiri[1] and Yusuke Nakamura[1,2]

[1]*Department of Human Genome Analysis, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 1-37-1 Kami-Ikebukuro, Toshima-ku, Tokyo 170 and* [2]*Laboratory of Molecular Medicine, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108*

To investigate the molecular basis of altered expression of the H-cadherin gene, we used polymerase chain reaction-single strand conformation polymorphism and DNA sequencing to examine the H-cadherin gene in 48 primary breast cancers in which loss of the long arm of chromosome 16 had been detected. We identified no mutations other than somatic 5-bp deletion within the coding region in a single tumor. The very low frequency of mutation found in these experiments suggests that H-cadherin is usually not a primary target for carcinogenesis in human breast cancers, and that reduction of its expression is likely to be a consequence of some other genetic event(s).

Key words: Breast cancer — H-Cadherin — Mutation analysis

Although molecular analysis has revealed a number of loci for putative tumor suppressor genes associated with sporadic breast cancers, only the *p53* gene is known to be mutated in a significant proportion of these tumors.[1,2] Recent studies have shown that loss of heterozygosity (LOH) on chromosome 16q is significantly frequent in sporadic breast cancers and that this feature is correlated with malignant phenotype and extent of metastasis.[3–5] Those data indicate the likely presence of one or more tumor suppressor genes on 16q whose loss or inactivation plays a significant role in the progression of breast cancer. The H-cadherin gene, a recently identified member of the cadherin superfamily of cell adhesion molecules, was isolated by subtractive hybridization, which compared its level of expression in normal versus tumor cells of the mammary gland.[6] This gene was localized to chromosome 16q24 and, moreover, its expression was shown to be significantly reduced in human breast-cancer cell lines as well as in primary cancers. Introduction of functional H-cadherin cDNA into a breast-cancer cell line resulted in a significant morphological change, i.e., to a normal cell-like phenotype, and markedly diminished cell growth. Other investigators reported that deletions at chromosome 16q24 in breast cancers, where H-cadherin is located, were correlated with distant metastasis.[7]

The cadherin family of molecules are functionally related transmembrane glycoproteins responsible for the Ca^{2+}-dependent cell-cell adhesion during morphogenesis in the embryo, and for the maintenance of normal tissue architecture throughout the life of an adult organism.[8] As cadherins are involved in cell-cell adhesion, decreased cellular adhesion or interaction though dysfunction or loss of cadherin molecules could enhance neoplastic progression as well as invasiveness of tumor cells.[9–11] These data suggest that H-cadherin may be a candidate for the putative tumor suppressor gene on 16q24. To investigate that possibility, we examined the H-cadherin gene for mutations in primary breast cancers.

Tumor and corresponding normal tissues were obtained from breast cancer patients during surgery in the Cancer Institute Hospital, Tokyo. Forty-eight cases showing LOH on 16q24 were selected. Of the 48 tumors 8 were papillotubular carcinoma, 21 were solid-tubular carcinoma, and 19 were scirrhous carcinoma. All patients gave their informed consent for the genetic study. Total RNAs were extracted from each tumor and corresponding normal tissues with ISOGEN (Nippon Gene, Tokyo), a procedure based on acid guanidine thiocyanate-phenol-chloroform extraction.[12] Reverse transcription was carried out as described elsewhere,[13] using 100 ng of total RNA.

The entire coding region of the H-cadherin gene was examined by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). Additionally, the coding sequence was divided into 9 parts and reverse transcription-PCR (RT-PCR) primers to amplify each part were designed so that an end portion of each amplified fragment overlapped the 5′ end of the next part. Sequences of the primers are shown in Table I. A 10-ng aliquot of the cDNA was amplified by each PCR. The conditions were: one cycle at 94°C (2 min); four cycles at 94°C (30 s), with annealing at 65°C (Tann) for 30 s and at 72°C for 30 s; four cycles with Tann = 63°C; four cycles with Tann = 60°C; and 20 cycles with Tann =

58°C. The buffer conditions were as described earlier.[14] When variant bands were detected in SSCP analyses, the appropriate PCR products were subcloned into pT7-Blue (Novagen, Wisconsin) and their nucleotide sequences were determined as previously described.[14] The expression levels of H-cadherin gene in the breast cancers used in this study and normal mammary epithelial cells were examined. Coding region part 5, containing a part of

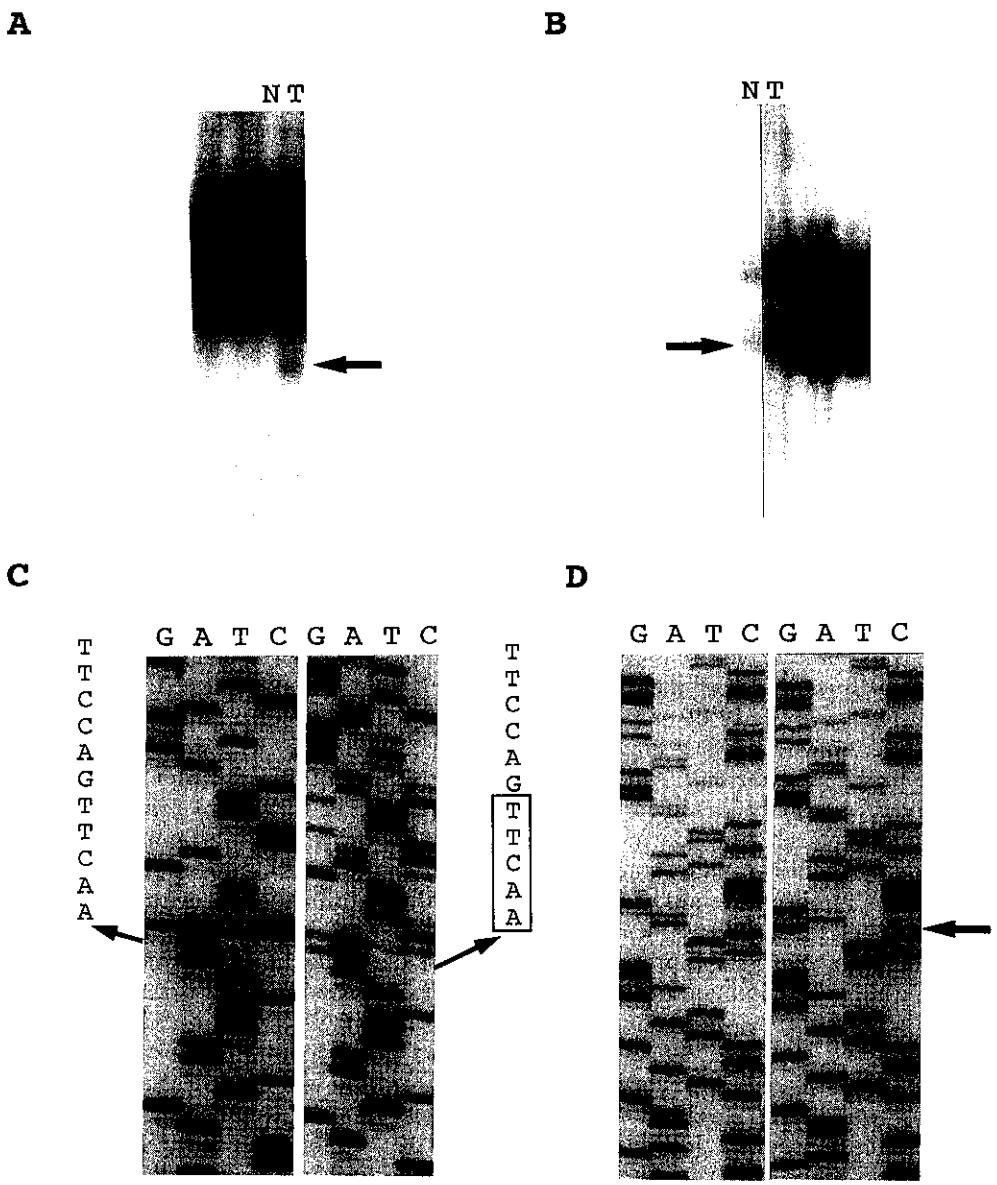

Fig. 1. Representative illustrations of PCR-SSCP and sequence analysis of the H-cadherin gene. A, B, amplification of coding region part 1 from patient 1626 (A) and patient 2632 (B). T, tumor DNA; N, corresponding normal DNA; arrows, aberrant bands. C, sequence analysis of PCR products of tumor and corresponding normal DNAs from patient 1626; sequences of the normal and mutated alleles are indicated on the left and right of the panel, respectively. A 5-bp deletion (AACTT) in part 1 was found in tumor DNA, indicated by the boxed nucleotides. Since a considerable number of subclones with a normal PCR product were pooled for the sequence template, the normal sequence was dominantly displayed in the tumor DNA sequence picture. D, sequence analysis of the relevant PCR product from patient 2632. A one-base substitution of A to G at codon 70 (arrow) was present in both tumor and corresponding normal DNAs (sequence on the right). Sequence on the left, normal control.

Table I. Primers Used for PCR Amplification of H-Cadherin[a)]

Amplicon	Nucleotide positions	Sense primer(5′→3′)	Antisense primer(5′→3′)
Part 1	181–480	AATGAAAACGCCGCCGGGCG	AGTTATGTTTCTCAGAGCAAC
2	431–720	ACTTCAAGGTGAACAGCGAT	TGGCCTGTCACTATCGACTA
3	681–1080	CCCAAGAGATGTTGGCAAGG	TTGACGGATATTATACCGCA
4	1041–1311	AGCCACCGATAATGCCCTCC	TTGGTGAATTTTGGTGAGTGA
5	1261–1560	GCCACGATCATGATCGATGAC	GTCTTCATTTTCCACTTTGA
6	1521–1798	TGCCTTCCACACCTGCTGA	GGTTAATATTCAGCCAACCTG
7	1749–2043	CATCAGGTATTCTGTTTACAAGGA	CTTATCTGATGCTCCCAAAATGA
8	1999–2280	GATGCCAAAAACCTCAGTGTAG	GTCCACTTTGGAATTCCTGC
9	2241–2520	CAGGGTACAAGTGTGCTCCT	TACAGACTAAGTGAAATTGT

a) Based on published sequences in reference 6.
Coding region: nucleotide 217–2358.

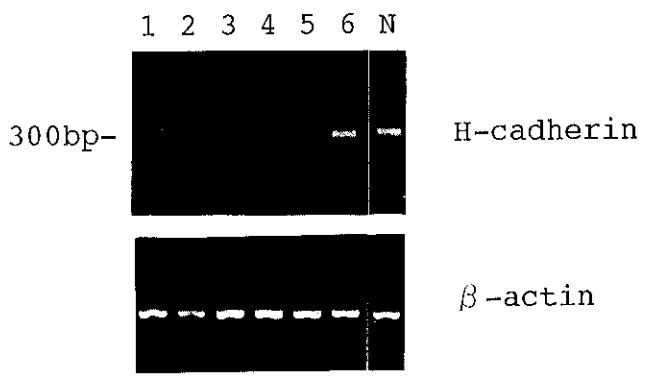

Fig. 2. RT-PCR analysis of H-cadherin expression in human normal mammary gland and breast cancers. Coding region part 5 of the H-cadherin gene was amplified by RT-PCR. Lanes 1–6, RNA from breast cancers. Lane N, RNA from human mammary gland (Clontech, California). The expression is reduced in lanes 1–5. β-Actin (838 bp) was used as a control.

cadherin domain II,[6] was amplified by RT-PCR. PCR conditions were the same as in the RT-PCR for SSCP, except that 10 cycles were applied with Tann = 58°C.

Fig. 1, A and B depict the aberrant SSCP patterns observed in two cases. DNA sequence analysis of the PCR products from patient 1626 revealed that tumor DNA had a 5-bp deletion (AACTT) at codons 53 and 54 (Fig. 1C), leading to a premature termination due to a frameshift. As this mutation was not present in the constitutional DNA, the alteration was considered to be a somatic event. In patient 2632, an A-to-G transversion at the third nucleotide of codon 70 was observed in both constitutional and tumor DNAs (Fig. 1D). Although this alteration was not observed in any other patient, it is likely to represent a rare polymorphism since it does not alter an amino acid. Reduced expression of H-cadherin in primary breast cancers was confirmed. In 5 of the 10 breast cancers examined the expression of H-cadherin was reduced as compared with that in normal mammary epithelial cells (Fig. 2). The expression in the other cases was not altered (only one case is shown).

LOH on the long arm of chromosome 16, being one of the most frequent genetic alterations observed so far in breast cancer, signals the presence of a tumor suppressor gene(s) on this chromosomal arm. At one time the E-cadherin gene on 16q22.1, a member of a subclass of the cadherin family that is involved in organization and maintenance of tissue structure, appeared to be a candidate for that tumor suppressor role[15,16] because reduced expression or dysfunction of E-cadherin is associated with a malignant phenotype and with invasive behavior of tumor cells.[17,18] Moreover, reduction of E-cadherin expression has been noted in poorly differentiated breast cancers[19] and mutations of the E-cadherin gene have been reported in some cases of lobular carcinoma of the breast.[20,21] However, as no E-cadherin mutations have been found in ductal carcinomas of the breast, inactivation of this gene may not be commonly involved in that type of tumor.[22]

The frequency of LOH at 16q24, where H-cadherin is located, is higher than that at 16q22-23; furthermore, chromosomal deletion at 16q24 is associated with distant metastasis in breast cancer.[7] The H-cadherin gene encodes a protein related to the cadherin superfamily of cell adhesion molecules; most of the key amino acids of the consensus sequence of cadherin domains are conserved, along with their putative Ca^{2+}-binding sites.

Expression of H-cadherin is significantly reduced in human breast carcinoma cell lines and in primary breast cancers. Other lines of evidence have also suggested that H-cadherin may be the sought-for candidate tumor suppressor gene at 16q24. In the present study, however, we detected only one somatic mutation among 48 breast cancers examined. Our results imply that somatic muta-

tion of H-cadherin is usually not one of the primary genetic events in human mammary carcinogenesis. Hence, the decreased expression of H-cadherin in breast cancers is likely to be caused by repression of transcription and/or translation of H-cadherin, in processes regulated by a gene that is a more favored target for mutation in breast cancers than the H-cadherin gene itself.

We thank Y. Nakajima and M. Minami for technical assistance, and Dr. F. Kasumi for providing clinical materials. This work was supported in part by a special grant for Strategic Advanced Research on Cancer from the Ministry of Education, Culture, Sports, and Science of Japan and by a grant from the Japanese Ministry of Health and Welfare.

(Received March 19, 1997/Accepted June 9, 1997)

REFERENCES

1) Cornelis, R. S., van Vliet, M., Vos, C. B. J., Cleton-Jansen, A.-M., van de Vijver, M. J., Peterse, J. L., Khan, P. M., Borresen, A. L., Cornelisse, C. J. and Devilee, P. Evidence for a gene on 17p13.3, distal to TP53, as a target for allele loss in breast tumors without p53 mutations. *Cancer Res.*, **54**, 4200–4206 (1994).
2) Devilee, P. and Cornelisse, C. J. Somatic genetic changes in human breast cancer. *Biochim. Biophys. Acta-Rev. Cancer*, **1198**, 113–130 (1994).
3) Sato, T., Akiyama, F., Sakamoto, G., Kasumi, F. and Nakamura, Y. Accumulation of genetic alterations and progression of primary breast cancer. *Cancer Res.*, **51**, 5794–5799 (1991).
4) Cleton-Jansen, A.-M., Moerland, E. W., Kuipers-Dijkshoorn, N. J., Callen, D. F., Sutherland, G. R., Hansen, B., Devilee, P. and Cornelisse, C. J. At least two different regions are involved in allelic imbalance on chromosome arm 16q in breast cancer. *Genes Chromosom. Cancer*, **9**, 101–107 (1994).
5) Tsuda, H., Callen, D. E., Fukutomi, T., Nakamura, Y. and Hirohashi, S. Allele loss on chromosome 16q24.2-qter occurs frequently in breast cancers irrespectively of differences in phenotypes and extent of spread. *Cancer Res.*, **54**, 513–517 (1994).
6) Lee, S. W. H-Cadherin, a novel cadherin with growth inhibitory functions and diminished expression in human breast cancer. *Nat. Med.*, **2**, 776–782 (1996).
7) Lindblom, A., Rotstein, S., Skoog, L., Nordenskjold, M. and Larsson, C. Deletions on chromosome 16 in primary familial breast carcinomas are associated with development of distant metastasis. *Cancer Res.*, **53**, 3707–3711 (1993).
8) Takeichi, M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science*, **251**, 1451–1455 (1991).
9) Takeichi, M. Cadherins in cancer: implications for invasion and metastasis. *Curr. Opin. Cell Biol.*, **5**, 806–811 (1993).
10) Behrens, J. The role of adhesion molecules in cancer invasion and metastasis. *Breast Cancer Res. Treat.*, **24**, 175–184 (1993).
11) Vleminckx, K., Vakaet, L., Mareel, M., Fiers, W. and von Roy, F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasive suppressor role. *Cell*, **66**, 107–119 (1991).
12) Chomczynski, P. A. Reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *BioTechniques*, **15**, 532–536 (1993).
13) Koyama, K., Sudo, K. and Nakamura, Y. Isolation of 115 human chromosome 8 specific expressed sequence tags by exon amplification. *Genomics*, **26**, 245–253 (1995).
14) Miki, Y., Katagiri, Y., Kasumi, F., Yoshimoto, Y. and Nakamura, Y. Mutation analysis in the BRCA2 gene in primary breast cancers. *Nat. Genet.*, **13**, 245–247 (1996).
15) Takeichi, M. Cadherins: molecular family important in selective cell-cell adhesion. *Annu. Rev. Biochem.*, **59**, 237–252 (1990).
16) Birchmeier, W., Behrens, J., Weidner, K. M., Frixen, U. H. and Schipper, J. Dominant and recessive genes involved in tumor cell adhesion. *Curr. Opin. Cell Biol.*, **3**, 832–840 (1991).
17) Behrens, J., Mareel, M. M., von Roy, F. M. and Birchmeier, W. Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *J. Cell Biol.*, **108**, 2435–2447(1989).
18) Frixen, U. H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., Loechner, D. and Birchmeier, W. E-Cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J. Cell Biol.*, **113**, 173–185 (1991).
19) Oka, H., Shiozaki, H., Kobayashi, K., Inoue, M., Tahara, H., Kobayashi, T., Takatsuka, Y., Matsuyoshi, N., Hirano, S., Takeichi, M. and Mori, T. Expression of E-cadherin molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res.*, **53**, 1696–1701 (1993).
20) Kanai, Y., Oda, T., Tsuda, H., Ochiai, A. and Hirohashi, S. Point mutation of E-cadherin gene in invasive lobular carcinoma of the breast. *Jpn. J. Cancer Res.*, **85**, 1035–1039 (1994).
21) Bert, G., Cleton-Jansen, A.-M., Nollet, F., de Leeuw, W. J. F., van de Vijver, M. J., Cornelisse, C. and van Roy, F. E-Cadherin is a tumor/invasion suppressor gene mutated in human lobular breast cancers. *EMBO J.*, **14**, 6107–6115 (1995).
22) Kashiwaba, M., Tamura, G., Suzuki, Y., Maesawa, C., Ogasawara, S., Sakata, K. and Satodate, R. Epithelial-cadherin gene is not mutated in ductal carcinomas of the breast. *Jpn. J. Cancer Res.*, **86**, 1054–1059 (1995).