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THE DCC PROTEIN EXPRESSION IN BREAST CARCINOMA

Yung-Hsiang Hsu and Cheng-Kuang Shaw*

Inactivation of the gene "deleted in colon cancer" (DCC) on chromosome 18 is known to be associated with the tumorigenesis and metastasis of colorectal cancer. In the present study, we investigated the expression of DCC, P53 and HER-2/neu product in surgical specimens from 79 patients with invasive breast cancer by immunohistochemistry staining and found the expression of DCC to be decreased in 42 tumors (52%). Overexpression of HER-2/neu and P53 was detected in 29 (36.8%) and 26 (32.9%) of this 79 breast cancer specimens, respectively. To evaluate the outcomes of the 79 breast cancer patients, we followed up the patients during the period from May 1990 to August 1998. The average length of follow-up was 52 months (ranging from 4 to 94 months). Patients with tumors having a combination of DCC-negative and HER-2/neu overexpression showed a marginal influence on survival time of breast cancer (P=0.06). However, patients with tumors having a combination of DCC-neagive and P53 overexpression showed no influence of these on survival time of breast cancer (P=0.36). These findings suggest that a decreased DCC expression and HER-2/neu overexpression may influence the prognosis of breast cancer.

Key words: DCC, HER-2/neu, P53, breast carcinoma

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The DCC (deleted in colon cancer) gene, located on chromosone 18 q 21.3 codes for a 190 KD transmembrane phosphoprotein with 42% homology to neural cell adhesion molecules (N-CAM), a fibronectin type Ⅲ related domain and four immunoglobulin domains of the C_2 class [1].

Genetic alterations are frequently associated with neoplasia. The activation of protooncogenes and the inactivation of tumor suppressor genes may be responsible for oncogenesis and many tumor suppressor genes have been isolated with a loss of heterozygosity (LOH) in several types of human neoplasia [2].

It has been reported that several chromosomal regions in breast cancer undergo a LOH in frequencies from 20% to 60% [3-5]. A LOH of 18q has been observed in approximately 40% of breast cancer patients [3,6]. These observations prompted us to investigate the relationship between the expression of the DCC, HER-2/neu, P53, Estrogen receptor(ER) and Progesterone receptor (PR) and the outcomes of 79 breast cancer patients, using immunohistochemical staining.

MATERIALS AND METHODS

Patients information

A total of 79 patients with invasive breast cancer were treated surgically at Tzu-Chi General Hospital between 1990 and 1993. Three advanced (stage Ⅳ) patients only received incisional biopsy. Others underwent modified radical mastectomy with axillary lymph nodes dissection, and all patients were followed up for a minimum of 5 years or until death. The average of follow-up interval was 52 months (ranging from 4 to 94 months). The following clinicopathologic fac-

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tors were recorded: age, tumor size and pathologic lymph node status.

Tumor tissues

Seventy-nine carcinomas of the breast stored as paraffinized tissue blocks in the archives of Tzu-Chi General Hospital were used in this study. Histological review confirmed 76 infiltrating ductal carcinoma, 2 infiltrating lobular carcinoma and one mucinous carcinoma.

Immunohistochemical staining

(A) Antibodies

a. Rabbit polyclonal anti-DCC antibody was a gift from Dr. Chuong [7]. Rabbit polyclonal anti-DCC antibody was developed using in vitro immunization with synthesis peptide as an antigen, as described previously [7]. The antigenic peptide was designed from codon 721 to 730 (DESVPDQPS) of third fibronectin type Ⅲ domain of DCC gene.

b. Rabbit polyclonal anti-HER-2/neu antibody, mouse monoclonal anti-ER, anti-PR and anti-P53 antibodies were ordered from the Dako company.

(B) Procedure

Individual tissue sections of 4 to 5 μ m were deparaffinized and heated in a 10 mM citric acid monophosphate buffer (pH 6.0) for 30 minutes in a 1.35-KW microwave oven at high power [8]. This method of enhancing the recognition of antigen in archival tissue is termed antigen retrieval. To minimize the evaporation of buffer during heating, the tissue slides were microwaved in a nommetalic kitchen pressure cooker. Immunohistochemical staining was performed with the labeled streptavidin of a complex kit (Dako, Japan). The immunohistochemistry technique involved the sequential application of the following antibodies: primary rabbit anti-DCC antibodies (1:300), mouse anti-ER antibodies (1:100), mouse anti-PR (1:50), rabbit anti-HER-2/neu antibody (1:200), mouse anti-P53 (1:100), a biotinylated anti-rabbit and anti-mouse 2nd antibodies (half hour) and a tertiary streptavidin peroxidase (half hour). Each antibody incubation was followed by rinsing the tissue sections in phosphate buffered saline three times (5 minutes, each).

Following treatment with a chromogen-AEC, the sites of immunoprecipitate formation were identified by light microscopy. Positive and negative control sections were included with each assay. Samples were to be regarded as positive for DCC when at least 25 percent of the tumor cells were granular cytoplasmic immunoreactive. However, this classification proved to be unnecessary, since staining for DCC turned out to be an "all-or-nothing" phenomenon [9]. Membrane staining was interpreted as HER-2/neu oncoprotein expression. The amount of staining was scored in a blinded fashion as negative (no immunostaining), trace positive (few, detectable immunostaining cells scattered through the tumor or located along one edge of the specimen), moderate immunostaining (distinct membrane staining in the majority of cells) or strong immunostaining (intense membrane staining in the majority of cells) [10]. Overexpression is identified immunohistochemically as moderate and strong membrane immunostaining, as previously described [11]. The immunostaining of ER, PR, P53 was regarded as positive when at least 25 percent of the tumor cells were nuclear immunoreactive.

Statistical analysis

The association between DCC expression in tumors and various prognostic factors were assessed by the chi-square test. Statistical analyses were performed using SAS software (Cary, NC, USA). Kaplan-Meier estimates were used to display survival among different patient groups. Differences between the two curves were tested statistically by the Log-rank test.

RESULTS

DCC protein product expressed in the surface of ductal epithelium in the normal breast tissue (Fig. 1A). In the invasive breast carcinomas, only 37(48%) of 79 breast cancer specimens showed postive staining for the DCC protein which is located in the cytoplasm of cancer cells(Fig. 1B). The overall survival of the patients with DCC-negative tumors was lower than of those with DCC-positive tumors but this difference was not significant (P=0.58) (Fig. 2). Overexpression of P53 was detected in the nucleus in 26 (32.9%) of the 79 breast cancers. DCC expression in tumors was associated with P53 overexpression (P=0.01). DCC expression of tumors was not related to any other standard prognostic factors (Table 1). Overexpression of HER-2/neu was detected in the membrane in 29 (36.8%) of 79 breast cancers, but no correlation with DCC positivity was found. However, 14 patients with DCC-negative and HER-2/neu overexpression tumors showed a marginal influence of these on the post-operation survival of breast cancer (P=0.06) (Fig. 3). A further 8 patients with DCC-negative and P53 overexpression tumors showed no influence on the post-operation survival of breast cancer (P=0.36) (Fig. 4).

Table 1. Relationship between the expression of DCC protein and clinicopathological factors in breast cancer patients

Prognostic	Factor	Number	DCC Positive N=37	DCC Negative N=42	P
Age	<=50	37	24	20	0.12
	>50	42	13	22	
Size	<=20mm	11	6	5	0.05
	21~50mm	47	17	30	
	>=51mm	21	14	7	
*Histo	Infiltrating	76	37	39	0.09
	Other type	3	0	3	
*LN	Negative	29	13	16	0.53
	1-3	14	5	9	
	2-4	36	19	17	
*ER	Negative	47	24	23	0.36
	Positive	32	13	19	
*PR	Negative	41	22	19	0.20
	Positive	38	15	23	
P53	Negative	53	19	34	0.01
	Positive	26	18	8	
HER-2/neu	Negative	50	22	28	0.50
	[1] Positive	29	15	14	

[1] positive: moderate and strong membrane staining
*Histo: histologic type, LN: axillary lymph node, ER: estrogen receptor, PR: progesterone receptor

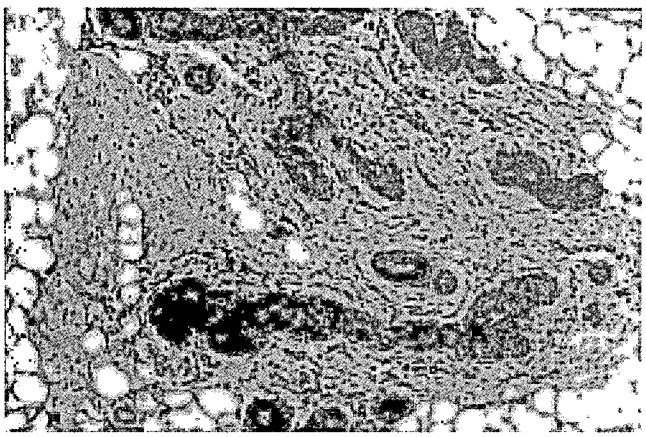

Fig. 1A. DCC protein expressed in the surface of normal ductal epithelial cells (x100)

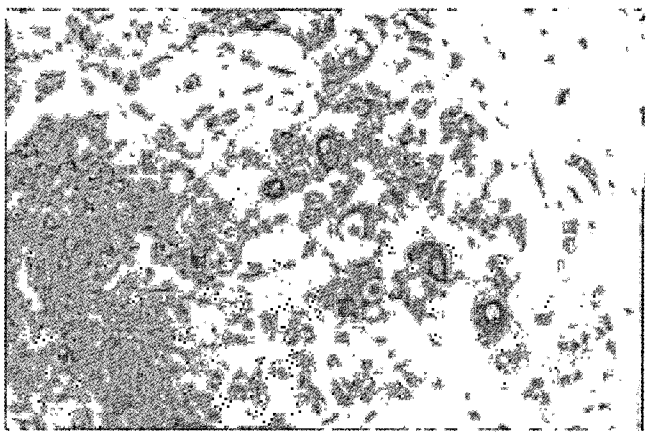

Fig. 1B. Invasive breast carcinoma showing positive cytoplasmic staining for DCC (x200)

DISCUSSION

The candidate tumor suppressor gene DCC was isolated by Fearon, who mapped the allelic deletion of chromosome 18q 21.3 in colorectal carcinoma [1]. DCC expression was reduced or absent in 88% of different cholorectal carcinoma cell lines and in 70% of colorectal cancers [1,12], which is consistent with a tumor suppressive function of the gene product. A decreased expression of DCC mRNA was observed in human colorectal cancers and found to be related to prognosis and distant metastasis [13,14]. Moreover,

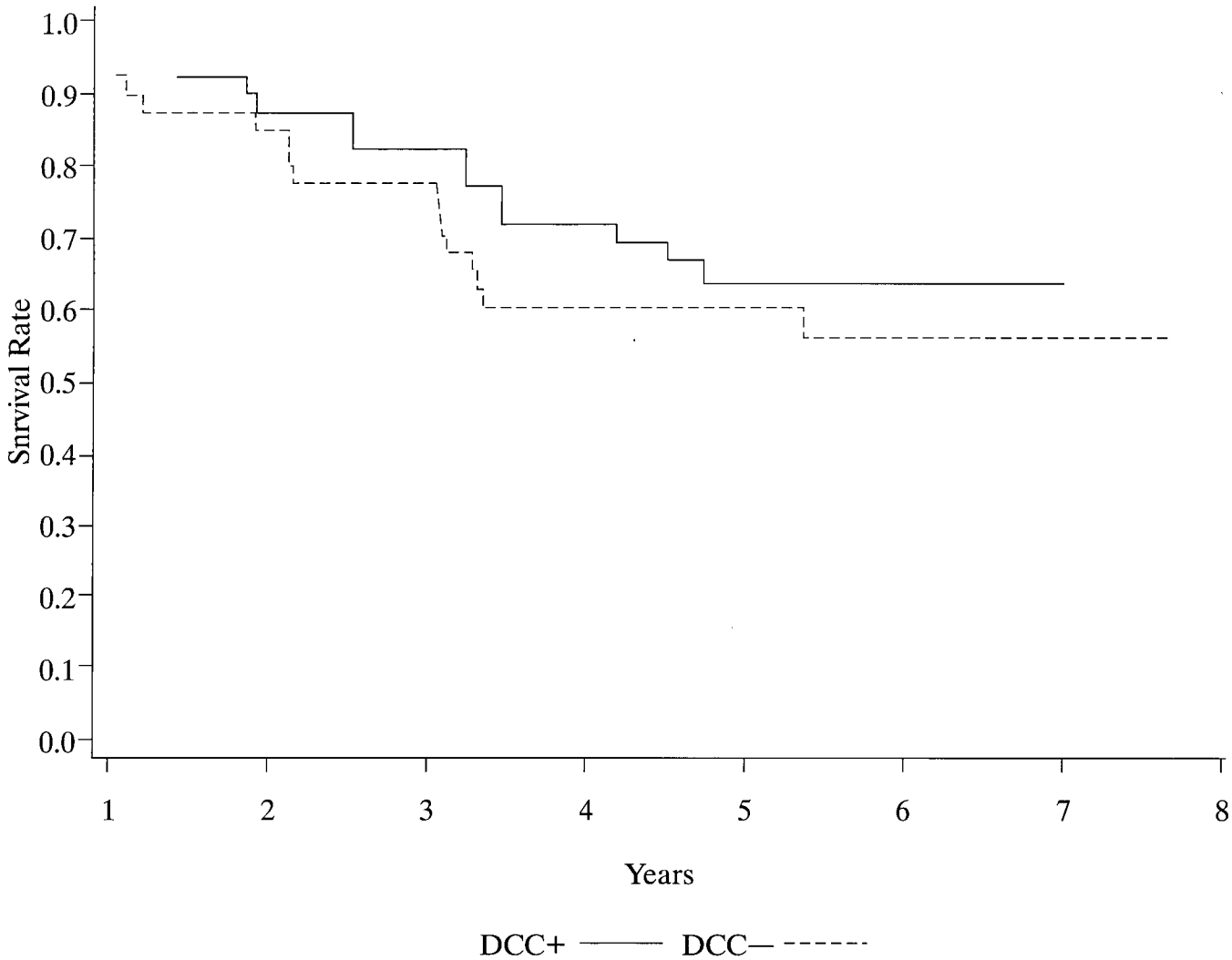

Fig. 2. Post-operation survival of patients with breast cancer, according to immunohistochemical staining of DCC in the primary tumor (Kaplan-Meier method)

frequent loss of the expression of DCC in pancreatic ductal adenocarcinoma and prostatic carcinoma [15, 16] and a LOH of 18q in stomach cancer and esophageal cancer have also been reported [17,18].

In breast cancer, several chromosomal regions have been reported to undergo LOH in frequencies of from 20% to 60% [3-5]. The LOH of 18q is reported in approximately 40% of breast cancers; however, the LOH of 18q does not always include the DCC locus. According to previous study, LOH at the DCC locus was deleted in 52% of patients with breast cancer, while 77% of those having DCC-LOH showed a distinct reduction or loss of DCC expression, using polymerase chain reaction [19]. Our immunohistochemical staining was found in approximately half of the breast cancers, which is in agreement with previous reports [20]. These findings indicate that DCC plays a role in the oncogenesis of breast cancer.

A genetic model stipulating that multistep alteration in oncogen and allelic losses are nesessary for colorectal tumorigenesis was advocated [21]. Concordant P53, DCC and c-ki-ras alteration are all known to be associated with tumorigenesis or metastasis of colorectal carcinoma. A variety of genetic change in breast carcinoigenesis, including the amplification of oncogense (HER-2/neu, int-2, c-myc, H-ras) and the deletion or mutation of the tumor suppressor genes (RB, P53) have been reported [22,23].

Although some of these oncogenes and suppuressor genes have been thought to be prognostic factors, their prognostic value is still controversial. However, coexpression of the oncogens was found to

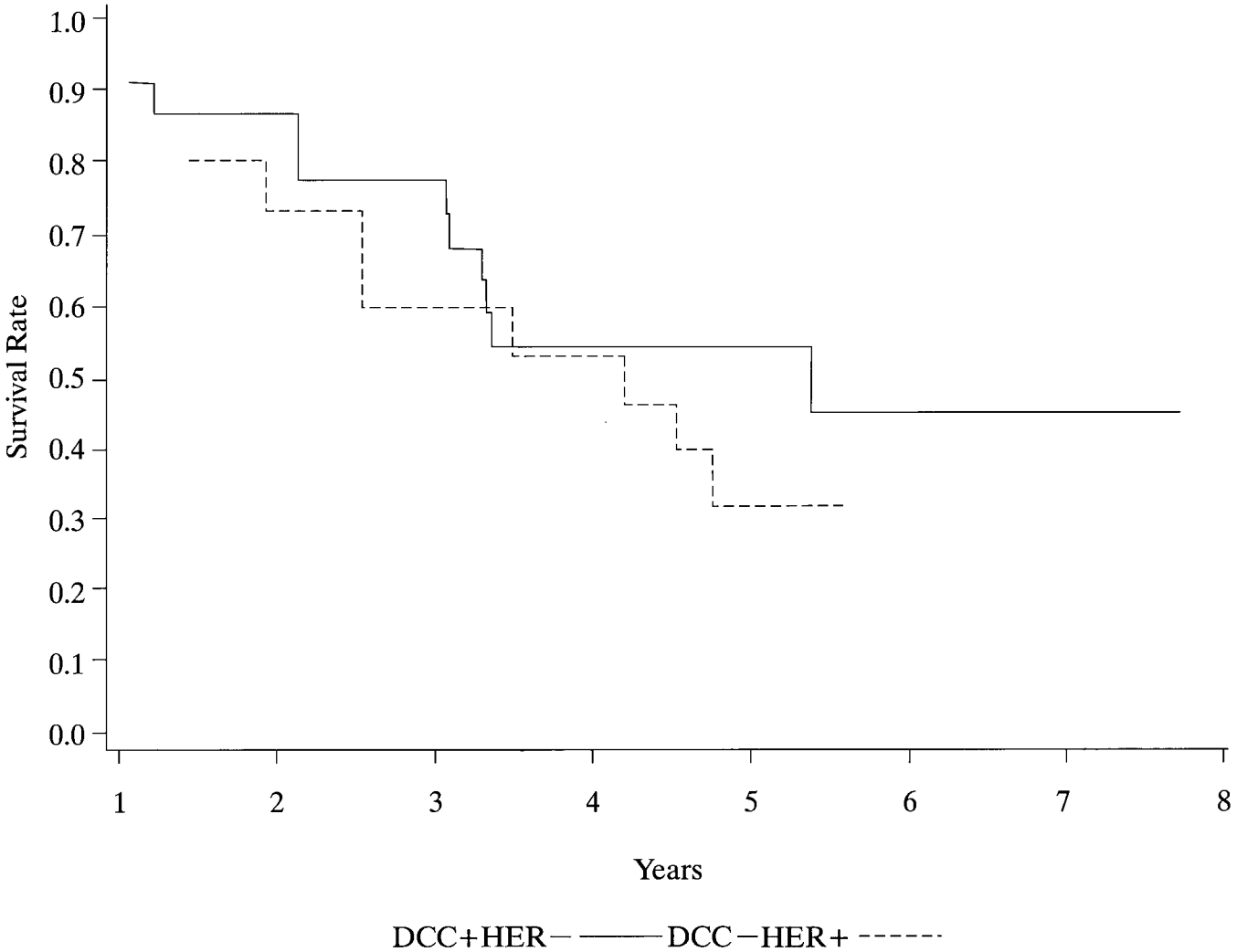

Fig. 3. Post-operation survival, according to the combination of DCC and HER-2/neu immunostaing (Kaplan-Meier method)

function as a strong prognostic correlation for recurrence and survival [23]. In this study, the single alteration of DCC, HER-2/neu or P53 did not possess independent prognostic significance for the prediction of survival, but patients having breast cancer with double alteration of DCC-negative and HER-2/neu overexpression showed that these had marginal influence of post-operation survival time of breast cancer. These data suggested that decreased DCC expression and HER-2/neu overexpression may influence the prognosis of breast cancer.

The DCC gene encodes a deduced protein with multiple immunoglobulin domains and fibronectin type Ⅲ repeats. The predictive amino acid sequence is highly simlar to Immunoglobulin (Ig) superfamily of cell adhesion molecules that includes N-CAM, fasciclin Ⅱ, L1, and others [1], whereas direct evidence that the gene encodes a cell adhesion molecular has not yet been obtained.

The homology of the DCC molecule to the cell adhesion molecule indicates that alteration of DCC expression influences the incidence of distant metastasis [13,14]. According to our data, the negative staining of DCC was associated with lower post-operative survival time, which suggested that the distribution of intracellular adhesion and communication play important roles in the genesis and/or progression of breast cancer. Although the biological function of the DCC gene has not been fully elucidated, further investigation of DCC genesis will provide adhesion molecules in carcinogenesis.

In conclusion, DCC expression was found in 48% of 79 patients and showed better outcomes than DCC negative patients of breast cancer. The DCC expres-

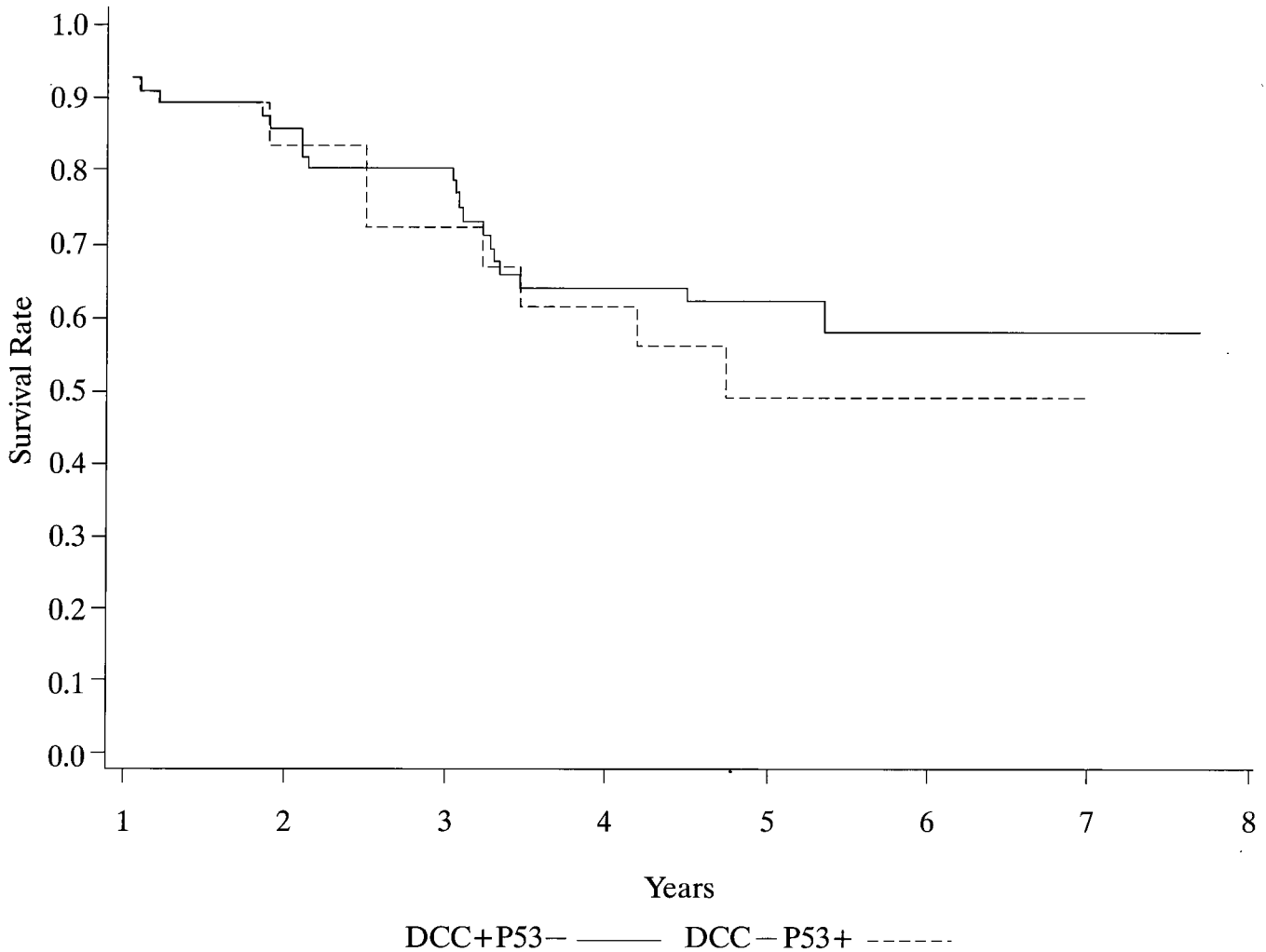

Fig. 4. Post-operation survival, according to the combination of DCC and P53 immunostaining (Kaplan-Meier method)

sion is associated with p53 overexpression. Double alteration of DCC-negative and HER-2/neu overexpression may influence the post-operative survival time.

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REFERENCES

1. Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, Thomas G, Kinzler KW, Vogelstein B. A genetic model for colorectal tumorigenesis. Identification of a chromosome 18q that is altered in colorectal cancers. Science 1990; 247: 49-56.
2. Weinberg RA. Tumor suppressor genes. Science 1991; 254: 1138-1145.
3. Callahan R, Cropp C, Merlo GR, Diella F, Venesio T, Lidereau R, Cappa APM, Lisicia DS. Genetic and molecular heterogeneity of breast cancer cells. Clin Chim Acta 1993; 217: 63-73.
4. Sato T, Akiyama F, Sakamoto G, Kasumi F, Nakamura Y. Accumulation of genetic alterations and progression of primary breast cancer. Cancer Res 1991; 51: 5794-5799.
5. Takita K, Sato T, Miyagi M, Watatani M, Akiyama F, Sakamoto G, Kasumi F, Abe R, Makamura Y. Correlation of loss of alleles on the short arms of chromosomes 11 and 17 with metastasis of primary breast cancer to lymph nodes. Cancer Res 1992; 52: 3914-3917.
6. Cropp C, Lidereau R, Champbell G, Champene MH, Callahan R. Loss of heterozygosity on chromosome 17 and 18 in breast carcinoma: two new regions

identified. Proc Natl Acad Sci USA 1990; 87: 7737-7741.
7. Chuong CM, Jiang TX, Yin E, Widelitz RB. cDCC (chicken homologue to a gene deleted in colorectal carcinoma) is an epithelial adhesion molecule expressed in the basal cells and involved in epithelial-mesenchymal interaction. Dev Biol 1994; 164: 383-397.
8. Brown RW, Chirala R. Utility of microwave-citrate antigen retrieval in diagnostic immunohistochemistry. Mod Pathol 1995; 8: 515-520.
9. Shibata D, Reale MA, Lavin P, Silverman M, Fearon ER, Steele G, Jessup JM, Loda M, Summerhayes IC. The DCC protein and prognosis in colorectal cancer. N Engl J Med 1996; 335: 1727-1732.
10. Press MF, Pike MC, Hung G, Jy, Ma Y, George J, Dietz-Band J, James W, Slamon DJ, Batsakis JG. Amplification and overexpression of HER-2/neu in carcinomas of the salivary gland: Correlation with poor prognosis. Cancer Res 1994; 54: 5675-5682.
11. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udore J, Ullrich A. Studies of HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244: 707-712.
12. Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Makamura Y, White R. Allelotype of colorectal carcinomas. Science 1989; 244: 207-211.
13. Itoh F, Hinoda Y, Ohe M, Ohe Y, Ban T, Endo T, Lmai K, Yachi A. Decreased expression of DCC mRNA in human colorectal cancers. Int J Cancer 1993; 53: 260-263.
14. Jen J, Kim H, Piantadosi S, Liu Z, Levitt RC, Sistonen P, Kinzier KW, Vogelstein B, Hamilton SR. Chromosome 18q loss and prognosis of colorectal cancer. N Engl J Med 1994; 331: 213-221.
15. Hohne MW, Halatsch ME, Kahl GF, Weinel RJ. Frequent loss of expression of the potential tumor suppuressor gene DCC in ductal pancreatic adenocarcinoma. Cancer Res 1992; 52: 2616-2619.
16. Gao X, Honn KV, Grignon D, Sakr W, Chen YQ. Frequent loss of expression and loss of heterozygosity of the putative tumor suppressor gene DCC in prostatic carcinomas. Cancer Res 1993; 53: 2723-2727.
17. Uchino S, Tsuda H, Noguchi M, Yokota J, Terada M, Saito T, Kobatashi M, Sugimura T, Hirohashi S. Frequent loss of heterozygosity at the DCC locus in gastric cancer. Cancer Res 1992; 52: 3099-3122.
18. Huang Y, Boynton RF, Blount PL, Silverstein RJ, Yin Y, Tong Y, McDaniel TK, Newkirk C, Resau JH, Sridhara R, Reid BJ, Meltzer SJ. Loss of heterozygosity involves multiple tumor suppuressor genes in human esophageal cancer. Cancer Res 1992; 52: 6525-6530.
19. Kashiwaba M, Tamura G, Ishida M. Frequent loss of heterozygosity at the deleted colorectal carcinoma gene locus and its association with histologic phenotypes in breast carcinoma. Virchows Arch 1995; 426: 441-446.
20. Wakita K, Kohno N, Sakoda Y, Ishikawa Y, Sakaue M. Decreased expression of the DCC gene in human breast carcinoma. Jpn J Surg 1996; 26: 900-903.
21. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1991; 61: 759-767.
22. Slamon DJ, Glark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: Correlation of relapse and survival with amplication of the HER-2/neu oncogene. Science 1987; 235: 177-182.
23. Bland KI, Konstadoulakis MM, Vezeridis MP, Wanebo HJ. Oncogene protein co-expression: Value of Haras, c-myc, c-fos, and P53 as prognostic discriminants for breast carcinoma. Ann Surg 1995; 211: 706-718.

DCC蛋白在乳癌之表達

許永祥 蕭正光*

DCC基因位於第十八對染色體上，此基因不活化與大腸直腸癌之發生與轉移有關。本研究，用免疫組織化學染色方法觀察79例侵襲性乳癌細胞內DCC蛋白，P53及HER-2/neu之表達。DCC蛋白表達減少者有42例（52%），而HER-2/neu及P53過度表現率分別為36.8% (29/79)及32.9% (26/79)。為了評估病人預後，病人追蹤時間由1990年5月到1998年8月，平均追蹤52個月（4~94個月），病人同時合併DCC陰性及HER-2/neu過度表現對乳癌預後有邊緣效應（P=0.060）。但病人同時合併DCC陰性及P53過度表現則對乳癌預後無影響（P=0.36）。本研究顯示同時DCC蛋白表現減少及HER-2/neu過度表現可能影響乳癌預後。

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