Decreased Expression of Tumor Protein p53-induced Nuclear Protein 1 (TP53INP1) in Breast Carcinoma

YASUHIRO ITO[1], YOSHIHARU MOTOO[2], HIROSHI YOSHIDA[1], JUAN LUCIO IOVANNA[3], YUUKI TAKAMURA[1], AKIHIRO MIYA[1], KANJI KUMA[1] and AKIRA MIYAUCHI[1]

[1]Kuma Hospital, Kobe; [2]Department of Internal Medicine and Medical Oncology, Cancer Research Institute, Kanazawa University, Kanazawa, Japan; [3]Centre de Recherche INSERM, EMI 0116, Marseille, France

Abstract. *Background: Tumor protein p53-induced nuclear protein 1 (TP53INP1) is a stress-induced protein and plays a role in cell cycle arrest and p53-mediated apoptosis. In this study, TP53INP1 expression in human breast carcinoma was investigated. Materials and Methods: TP53INP1 and aberrant p53 expression were investigated immunohistochemically in 81 cases of breast carcinoma. Results: Diffuse and intense TP53INP1 expression was observed in the normal mammary gland. Decreased TP53INP1 expression was found in 45 cases (55.6%) of breast carcinoma. The TP53INP1 expression level was inversely linked to tumor size, positive lymph node metastasis, high histological grade and aberrant p53 expression. Conclusion: Decreased expression of TP53INP1 is involved in breast carcinoma progression.*

The *tumor protein 53-induced nuclear protein 1* (*TP53INP1*) gene, one of the *p53* target genes, is a tumor suppressor gene located on the chromosome 8q22 (1). Originally, the transcription of this gene was induced in mice with acute pancreatitis (2) and also in cell lines under exposure to stress agents (3, 4). This gene encodes two protein isoforms, TP53INP1alpha and TP53INP1beta, which interact with p53 and the serine-threonine p53-kinase HIPK2 within the promyelocytic leukemia protein nuclear bodies (5). These two isoforms also modify the transcriptional activity of p53 on some target genes, such as *p21* and *mdm2* (3). Furthermore, they regulate p53-mediated apoptosis and cell cycle arrest in the G1-phase induced by cellular stresses (3).

Recently, expression of p8, another stress-induced protein, has been shown in some human carcinomas, such as pancreatic carcinoma (6, 7), thyroid carcinoma (8, 9) and breast carcinoma (10), and it plays various roles in carcinoma progression in a tissue-specific manner. Also, expression of TP53INP1 was studied in gastric carcinoma and its expression level was decreased in cases showing aggressive characteristics (11), but this issue has not been studied in depth for other carcinomas. In this study, TP53INP1 expression in breast carcinoma, the most common malignancy originating from the endocrine organs, was investigated in order to confirm whether and how TP53INP1 is related to the biological aggressiveness and aberrant p53 expression.

Correspondence to: Yasuhiro Ito, MD, Ph.D., Department of Surgery, Kuma Hospital, 8-2-35, Shimoyamate-Dori, Chuo-ku, Kobe City, 650-0011 Japan. Tel: +81 78 371 3721, Fax: +81 78 371 3645, e-mail: ito01@kuma-h.or.jp

Key Words: TP53INP1, p53, breast carcinoma, immunohistochemistry.

Materials and Methods

Tissue specimens. Tissue specimens of breast carcinomas were obtained from 81 patients who underwent surgery in Kuma Hospital, Kobe, Japan, between 1999 and 2004. They consisted of five non-invasive ductal carcinomas, four invasive lobular carcinomas and 72 invasive ductal carcinomas. This project was approved by the ethics committee of our hospital and written informed consent was obtained from the participating patients. The tissues were fixed with 10% formalin and were paraffin-embedded for immunohistochemistry.

Immunohistochemistry. A standard avidin-biotin-peroxidase complex method (ABC) was adopted for TP53INP1 immunostaining using the ABC staining system kit (Santa Cruz Biotechnology, Santa Cruz CA, USA). Briefly, tissue sections 4-μm-thick were dewaxed and the endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 15 min. For antigen retrieval, sections were immersed in 0.03 mol/L citrate buffer (pH 6.0) and incubated at 95˚C for 40 min. After rinsing in phosphate-buffered saline (PBS) pH 7.2, 10% bovine serum (Wako, Osaka, Japan) was applied for 20 min to block non-specific reactions. The sections were then incubated overnight at 4˚C with 6 mg/ml of rat anti-human monoclonal antibody against TP53INP1 primary antibody, which recognizes both TP53INP1alpha and TP53INP1beta. This antibody was produced by our coworkers. After rinsing in PBS, the specimens were treated with biotinylated goat anti-rat IgG for 30 min, and avidin-biotin-HRP for 30 min. After washing with PBS, the peroxidase reaction was visualized by incubating the sections with 0.02% 3,3'-diaminobenzidine tetrahydrochloride in 0.05 M Tris-buffer with 0.01% hydrogen peroxide. The sections were counterstained with hematoxylin. Normal IgG was used as a negative control.

Table I. *Relationship between TP53INP1 expression and clinicopathological parameters in breast carcinoma.*

	TP53INP1 expression (%)			
	Negative or weak	Moderate	Strong	Total
Histology				
Non-invasive ductal	1 (20.0)	0	4 (80.0)	5
Invasive ductal	24 (33.3)	14 (19.4)	34 (47.2)	72
Invasive lobular	1 (25.0)	1 (25.0)	2 (50.0)	4
		Not significant		
Tumor size				
≥2 cm	18 (41.9)	9 (20.9)	16 (37.2)	43
<2 cm	8 (21.0)	6 (15.8)	24 (63.2)	38
		*p=0.0264		
Lymph node metastasis				
Positive	11 (42.3)	8 (30.8)	7 (26.9)	26
Negative	14 (29.8)	7 (14.9)	26 (55.3)	47
		*p=0.0272		(8 unknown)
Estrogen receptor				
Positive	19 (30.2)	13 (20.6)	31 (49.2)	63
Negative	6 (42.9)	2 (14.3)	6 (42.9)	14
Not significant		(4 unknown)		
Progesterone receptor				
Positive	13 (25.5)	9 (17.6)	29 (56.9)	51
Negative	7 (28.0)	6 (24.0)	12 (48.0)	25
		Not significant		(5 unknown)
Aberrant p53 expression				
Positive	9 (52.9)	2 (11.8)	6 (35.3)	17
Negative	17 (26.6)	13 (20.3)	34 (53.1)	64
		**p=0.0384		
Histological grade				
I	5 (16.7)	6 (20.0)	19 (63.3)	30
II	9 (36.0)	6 (24.0)	10 (40.0)	25
III	11 (52.4)	3 (14.3)	7 (33.3)	21
		***p=0.0321		
Menopausal status				
Pre-menopausal	15 (34.9)	11 (25.6)	17 (39.5)	43
Post-menopausal	11 (28.9)	4 (10.5)	23 (60.5)	38
		Not significant		

*Strong *vs.* others, ** Negative or weak *vs.* others, ***Grade I *vs.* II, III.

Immunostaining for p53 was peformed using a simple staining method kit (Nichirei, Tokyo, Japan). An anti-p53 monoclonal antibody (DO-7, Dako, Copenhagen, Denmark) was applied as a primary antibody at a concentration of 1:50.

Immunohistochemical evaluation. TP53INP1 immunoreactivity was observed mainly in the cytoplasm of cells. Nuclear staining was also observed. The cells were regarded as positive for TP53INP1 when immunoreactivity was clearly observed in their nuclei and/or cytoplasm, as described previously (11). The labeling index (LI) for each case was calculated by counting cells expressing these proteins per 1,000 cells. Cases were classified into four categories based on the TP53INP1 LI and staining intensity: negative, no positive cells could be found; weak, less than 10% of cells were positive; moderate, less than 50% of cells were intensely positive or, even if the LI exceeded 50%, only obscure staining could be detected; strong, 50% or more of cells were intensely positive.

For p53 immunostaining, cells were regarded as positive when nuclear immunostaining was clearly observed. Cases were regarded as showing aberrant p53 expression when the p53 LI was 10% or higher.

Statistical analyses. The Chi-square test and Fischer's exact test were employed to analyze the relationship between TP53INP1 expression and various clinicopathological features. *P*-values less than 0.05 were regarded as significant.

Results

In the normal mammary gland, diffuse and intense TP53INP1 expression was observed predominantly in glandular epithelial cells (Figure 1a). TP53INP1 expression in 81 cases of breast carcinoma was then studied and each case was

classified into one of the four categories, as described in Materials and Methods. Out of the 81 cases of breast carcinoma, 10 cases were negative, 16 cases weak, 15 cases moderate, and 40 cases strong, indicating that the TP53INP1 expression level was decreased in 41 cases (50.6%). Figure 1b shows the typical staining profile of TP53INP1 in breast carcinoma, which was classified as strong, and negative TP53INP1 expression is shown in Figure 1c. The relationship between TP53INP1 expression and the clinicopathological parameters of the 81 cases of breast carcinoma are shown in Table I. The TP53INP1 expression level was reduced in 20.0% of non-invasive ductal carcinoma, 52.8% of invasive ductal carcinoma and 50.0% of invasive lobular carcinoma. Furthermore, its expression level was more frequently decreased in cases showing tumor measuring 2 cm or larger, positive lymph node metastasis, or higher histological grade [Nottingham modification (12)]. The estrogen receptor status, progesterone receptor status, and menopausal status were not linked to TP53INP1 expression.

Aberrant p53 expression was not observed in normal mammary glands. However, this phenomenon could be seen in 17 out of 81 cases of breast carcinoma (21.0%) (Figure 1d) and showed a direct relationship with histological grade (data not shown). Furthermore, as shown in Table I, it was inversely correlated with TP53INP1 expression.

Discussion

In this study, it was demonstrated that: i) TP53INP1 was diffusely expressed in normal mammary glands, especially in glandular epithelial cells; ii) decreased TP53INP1 expression level was more frequently observed in cases showing a larger tumor, lymph node metastasis, or higher histological grade and iii) TP53INP1 expression level was inversely related to aberrant p53 expression.

A previous study has demonstrated that TP53INP1 expression was expressed in normal epithelial cells in the gastric mucosa (11). In this study, TP53INP1 was diffusely expressed in the normal mammary gland, indicating that this protein plays a constitutive role in many normal epithelial cells. However, since TP53INP1 was only occasionally expressed in human follicular epithelia of the thyroid (our unpublished data), TP53INP1 is apparently not distributed in all human glands. In the early phase of breast carcinoma, a high expression level of TP53INP1 was frequently detected, while the expression level significantly decreased in advanced cases. It has, therefore, been suggested that lack of TP53INP1 is significantly linked to breast carcinoma progression. Since TP53INP1 prevents cells from proliferating and mediates p53-induced apoptosis (3), TP53INP1-negative carcinoma cells should be more likely to be mitotic and less likely to become apoptotic, suggesting that this protein acts as a suppressor of breast carcinoma progression.

Previous studies have demonstrated that aberrant p53 expression is linked to aggressive characteristics of breast carcinoma, including poor prognosis (13, 14). In our series, aberrant p53 expression was directly related to histological grade, which is not discrepant with previous findings. Since the wild-type p53 is unstable and rapidly metabolized, cells having wild-type p53 are negative for p53 immunostaining. However, mutant p53 showed a longer half-life, resulting in immunohistochemical positivity (15). The inverse relationship between aberrant p53 expression and TP53INP1 expression level indicates that the TP53INP1 expression level was higher in cases showing wild-type p53. This finding seems reasonable because TP53INP1 can be transcriptionally induced by wild-type p53 (3, 4). However, 35% of cases showing aberrant p53 expression showed a strong expression of TP53INP1, indicating that TP53INP1 can also be induced in a p53-dependent manner. TP53INP1 expressed in cases showing aberrant p53 expression may play a role in negatively regulating the cell cycle or positively regulating apoptosis independently of the p53 status.

In summary, it was demonstrated that lack of or reduced expression of TP53INP1 is significantly related to breast carcinoma progression. Further studies regarding the mechanism of the induction of TP53INP1 expression in breast carcinoma especially in a p53-independent pathway are necessary to understand the role of this protein in breast carcinoma.

References

1 Nowak J, Tomasini R, Mattei MG, Azizi Samir LA, Dagorn JC, Dusetti N, Iovanna JL and Pebusque MJ: Assignment of tumor protein p53 induced nuclear protein 1 (TP53INP1) gene to human chromosome band 8q22 by *in situ* hybridization. Cytogenet Genome Res *97*: 140E, 2002.

2 Tomasini R, Pebusque MJ, Dagorn JC, Iovanna JL and Dusetti NJ: Molecular and functional characterization of the stress-induced protein (SIP) gene and its two transcripts generated by alternative splicing. SIP is induced by stress and promotes cell death. J Biol Chem *276*: 44185-44192, 2001.

3 Tomasini R, Samir AA, Carrier A, Isnardon D, Cecchinelli B, Soddu S, Malissen B, Dagorn JC, Iovannna JL and Duseti NJ: TP53INP1 and homeodomain-interacting protein kinase-2 (HIPK2) are partners in regulating p53 activity. J Biol Chem *278*: 37722-37729, 2003.

4 Okumura S, Arakawa H, Tanaka T, Nakanishi H, Ng CC, Taya Y, Monden M and Nakamura Y: p53DINP1, a p53-inducible gene, regulates p53-dependent apoptosis. Mol Cell *8*: 85-94, 2001.

5 D'Orazi G, Cecchinelli B, Bruno T, Manni I, Higashimoto Y, Saito S, Gostissa M, Coen S, Marchetti A, Del Sal G, Piaggio G, Fanciulli M, Appella E and Soddu S: Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. Nat Cell Biol *4*: 11-19, 2002.

6 Su SB, Motoo Y, Iovanna JL, Xie MJ, Mouri H, Ohtsubo K, Yamaguchi Y, Watanabe H, Okai T, Matsubara F and Sawabu N: Expression of p8 in human pancreatic cancer. Clin Cancer Res *7*: 309-313, 2001.

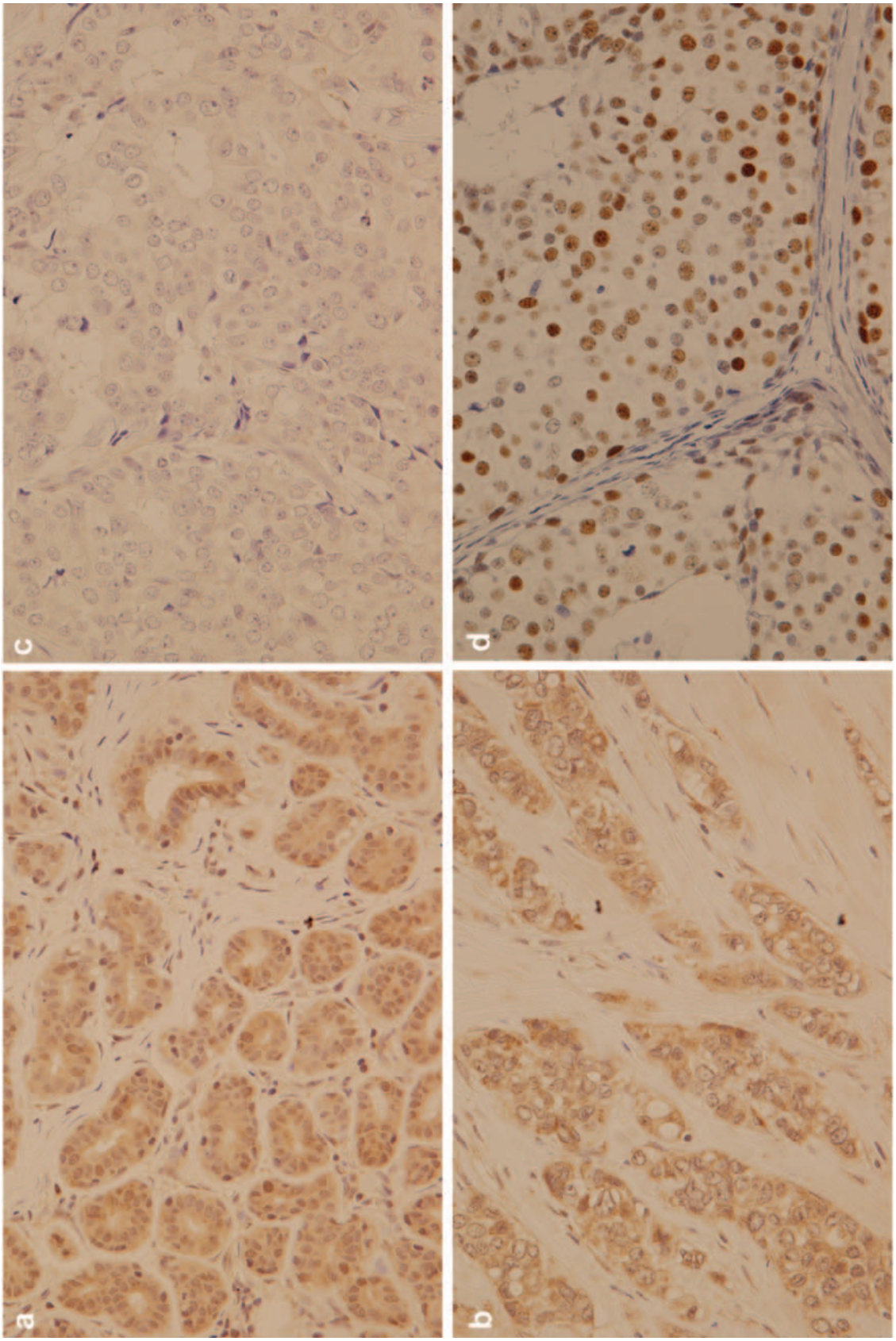

Figure 1. *a) Diffuse and intense TP53INP1 expression in normal mammary glands. b) Diffuse expression of TP53INP1 in breast carcinoma. c) Lack of TP53INP1 expression in breast carcinoma. d) Aberrant p53 expression in breast carcinoma. Original magnification; x250.*

7 Su SB, Motoo Y, Iovanna JL, Berthezene P, Xie MJ, Mouri H, Ohtsubo K, Matsubara F and Sawabu N: Overexpression of p8 is inversely correlated with apoptosis in pancreatic cancer. Clin Cancer Res *7*: 1320-1324, 2001.

8 Ito Y, Yoshida H, Motoo Y, Miyoshi E, Iovanna JL, Tomoda C, Uruno T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Matuura N, Kuma K and Miyauchi A: Expression and cellular localization of p8 protein in thyroid neoplasms. Cancer Lett *201*: 237-244, 2003

9 Ito Y, Yoshida H, Motoo Y, Iovanna JL, Tomoda C, Uruno T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Kuma K and Miiyauchi A: Expression of p8 protein in medullary thyroid carcinoma. Anticancer Res *25*: 3419-3424, 2005.

10 Ito Y, Yoshida H, Motoo Y, Iovanna JL, Nakamura Y, Kakudo K, Uruno T, Takamura Y, Miya A, Noguchi S, Kuma K and Miyauchi A: Expression of p8 protein in breast carcinoma; an inverse relationship with apoptosis. Anticancer Res *25*: 833-838, 205.

11 Jiang PH, Motoo Y, Garcia S, Iovanna JL, Pebusque MJ and Sawabu N: Down-expression of tumor protein p53 induced nuclear protein 1 in human gastric cancer. World J Gastroenterol *12*: 691-696, 2006.

12 Elston CW and Ellis IO: Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology *19*: 403-410, 1991.

13 Eissa S, Khalifa A, el-Gharib A, Salah N and Nohamed MK: Multivariate analysis of DNA ploidy, p53, c-erbB-2 proteins, EGFR, and steroid hormone receptors for prediction of poor short term prognosis in breast cancer. Anticancer Res *17*: 1417-1423, 1997.

14 Horne GM, Anderson JJ, Tianiakos DG, McIntosh GG, Thomas MD, Angus B, Henry JA, Lennard TW and Horne CH: p53 protein as a prognostic indicator in breast carcinoma: a comparison of four antibodies for immunohistochemstry. Br J Cancer *73*: 29-35, 1996.

15 Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M and Levine AJ: Activating mutations for transformation by p53 produced a gene product that forms an hsc70-p53 complex with an altered half-life. Mol Cell Biol *8*: 531-539, 1988.

Received July 25, 2006
Revised October 16, 2006
Accepted October 20, 2006