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Expression of p53 protein in benign epithelial hyperplasia, atypical ductal hyperplasia, non-invasive and invasive mammary carcinoma: an immunohistochemical study

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Abstract To clarify whether p53 protein expression is involved in multistep carcinogenesis or the progression of mammary ductal carcinoma, we investigated p53 protein expression in 83 invasive ductal carcinomas (IDC), 10 IDC with a predominant intraductal component, 13 non-invasive ductal carcinoma (NIDC), 16 atypical ductal hyperplasia (ADH) and 39 benign epithelial hyperplasia (EH), using immunohistochemistry. Expression of p53 protein was detected in 24 (28.9%) cases of IDC, 5 (50%) cases of IDC with a predominant intraductal component and 1 (7.6%) case of NIDC. No expression was observed in either ADH or EH. In IDC, including cases with a predominant intraductal component, p53 protein expression was associated with a higher histological grade ($P<0.0001$) or mitotic index ($P<0.0005$). Although overexpression of c-erbB-2 protein has also shown a similar association with these prognostic indicators, expression of p53 protein correlated regardless of the status of c-erbB-2 overexpression. Completely coordinated expression of p53 protein was seen in both intraductal and invasive components. The intraductal component in IDC including cases with a predominant intraductal component which expresses p53 protein had significantly higher histological grade ($P<0.0005$) or more comedo-subtypes ($P<0.0001$). These results suggested that p53 protein expression occurs at a stage of NIDC with high histological grade or in comedo-subtypes. Its expression is maintained throughout invasion.

Key words p53 · Immunohistochemistry · Breast cancer

Introduction

Mutations or abnormal expression of *p53* gene are common in a wide variety of human cancers, including breast cancer (Hollstein et al. 1991; Levine et al. 1991). Non-invasive ductal carcinoma (NIDC) is the earliest recognizable stage of breast cancer and it is widely assumed that intraductal disease gives rise to invasive cancer. Invasive ductal carcinoma (IDC) with a predominant intraductal component is also considered to represent an early stage of breast cancer progression. Atypical ductcal hyperplasia (ADH) is considered to confer an increased risk of breast cancer development (4–5 times that of a comparable group), and moderate or florid hyperplasia to confer a 1.5–2.0 fold increase in risk (Page 1992). We sought to clarify whether p53 protein expression is involved in multistep carcinogenesis or the progression of ductal carcinoma. As overexpression of c-erbB-2 protein is also considered to be important for progression or in determing the aggressiveness of mammary carcinoma, we also investigated overexpression of c-erbB-2 protein.

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Materials and methods

We used to following antibodies. CM-1 (Novocastra Laboratories, Newcastle, UK) is a polyclonal antibody raised against recombinant human p53 protein produced in a bacterial expression system (Midgley et al. 1992) which recognizes both the wild and mutant forms of the p53 protein. It can be used in formalin-fixed, paraffin-embedded tissue. DAKO-Collagen IV (CIV22) is a mouse monoclonal antibody raised against purified pepsin fragments of human type IV collagen isolated from human kidneys (Odermatt et al. 1984). Overexpression of c-erbB-2 protein was detected using a polyclonal antibody (Nichirei, Tokyo, Japan) to the internal domain of the gp185 (Mori et al. 1987).

Tissue samples of primary breast cancer and axillary lymph nodes were obtained from 106 Japanese female patients who underwent mastectomies at the SAGARA Hospitals (Kagoshima, Japan) between November 1989 and July 1993. The specimens included 13 NIDC, 10 IDC with a predominant intraductal component in which the amount of invasive carcinoma was less than one fourth that of the intraductal component (World Health Organization, 1981) and 83 IDC. All NIDC and IDC with a predominant intraductal component were diagnosed only in completely sectioned specimens. Invasion was determined by the absence of a basement membrane assessed by the immunohistochemical detection of collagen type IV (Fig. 3). ADH (16 patients), moderate or florid hyperplasia (20 patients) and mild hyperplasia (19 patients) were obtained by biopsy or mastectomy specimens coincident

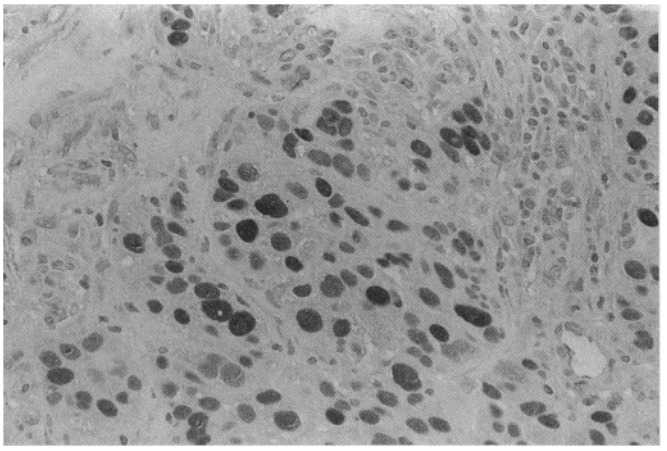

Fig. 1 Invasive component of an invasive ductal carcinoma showing nuclear staining for p53 (CM1), × 380

with IDC, NIDC, mastopathy or intraductal papilloma, and diagnosed according to the criteria of Page and Anderson (1987). Pieces of tumour and axillary lymph nodes were fixed in 10% neutrally buffered formalin for 24–48 h, and embedded in paraffin. Sections 3 μm thick were stained with haematoxylin and eosin, and were examined histopathologically. The mitotic index was defined as the total number of mitoses in ten high power fields (400× magnification) as previously described (Baak et al. 1985). Histological grade was determined using a modification of the method of Bloom and Richardson (1957).

For immunocytochemistry sections (3 μ m) were cut, mounted on poly-*L*-lysine-coated slides (Matsunami, Japan), air-dried overnight at room temperature (Midgley et al. 1992), dewaxed, rehydrated and stained with standard avidin-biotin-immunoperoxidase assays using the Vectastain ABC kit (Vector Laboratories, Burlingame, Calif., USA). CM-1 was used at dilution of 1: 1000, CIV22 and c-erbB-2 were 1: 100 and incubated overnight at +4 °C. Diaminobenzidine (0.03% in phosphate buffered saline) was used as the chromogen. Negative control sections were processed immunohistochemically without the primary antiserum (replaced by phosphate buffered saline containing 1.0% bovine serum albumin). Positive control sections were from breast tumours known to be stained positively for CM-1 antibodies. Immunostaining was performed twice and reproducible results were obtained.

Oestrogen receptor levels were determined using dextran coated charcoal separation (Biomedical Laboratories, Tokyo, Japan). A concentration greater than 14 fmol/mg of protein was considered positive.

The association of p53 protein expression with tumour size, histological grade, TNM stage, nodal status and mitotic index was analysed by means of the x^2 test.

Results

Nuclear staining of cells was considered positive for p53 protein expression (Fig. 1). The percentage of p53-positive cells was estimated semiquantitatively, and tumours were assigned into two categories: positive or negative. The number of samples and the rates of the positive cells were as follows: 10–20% positive cells in 4 samples; 20–50% positive cells in 9 samples, more than 50% in 16 samples. No nuclear staining was seen in any of the negative control sections, and surrounding non-cancerous breast tissue. The expression of p53 protein was observed in 29 of 93 samples (31.1%) of IDC including that with a predominant intraductal component. There was a statistically significant association between p53 protein expression and higher histological grade ($P<0.0001$), or mitotic index ($P<0.0005$) regardless of

Table 1 Association between p53 nuclear staining with CM1 and clinicopathological variables (*NS* not signifant)

Variable	Number of p53 positive cases (numbers in parentheses are c-erbB-2 negative cases	Number of p53 negative cases (c-erbB-2 negative)	*P* value
Tumor size (mm):			
0–20	7(6)	14(12)	NS
21–50	18(14)	34(30)	
51–100	4(2)	16(13)	
Lymph node metastasis:			
positive	10(8)	36(29)	NS
negative	19(14)	28(26)	
Oestrogen receptor:			
positive	11(9)	35(30)	NS
negative	19(13)	29(25)	
TNM stage:			
I and II	22(18)	43(40)	NS
III and IV	7(4)	21(15)	
Histological grade:			
I	0(0)	23(23)	$P<0.0001$
II	17(15)	33(29)	($P<0.0001$)
III	12(7)	8(3)	
Mitotic index:			
0–9	9(8)	49(44)	$P<0.0005$
10–20	9(6)	8(7)	($P<0.0001$)
>20	11(8)	7(4)	
Total	29(22)	64(55)	

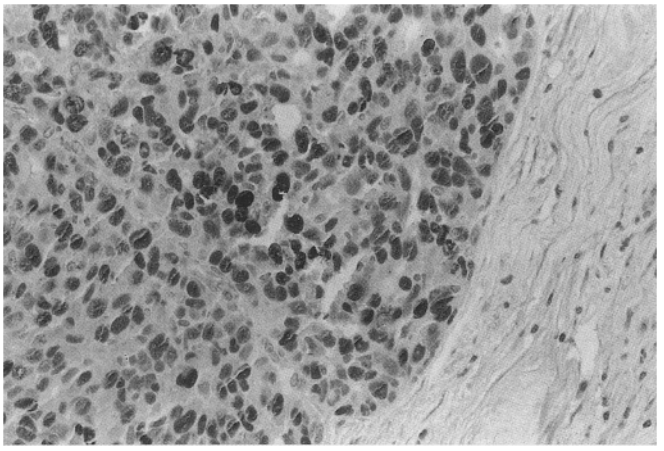

Fig. 2 Intraductal component of same patients in Fig. 1 showing nuclear staining for p53 (CM1), × 380

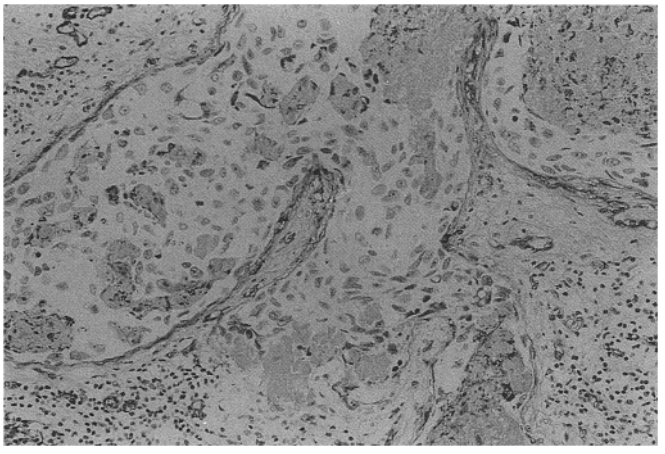

Fig. 3 Invasive ductal carcinoma with a predominant intraductal component showing partial depletion of basement membrane in which no immunoreactivity is noted for collagen IV antibody, × 250

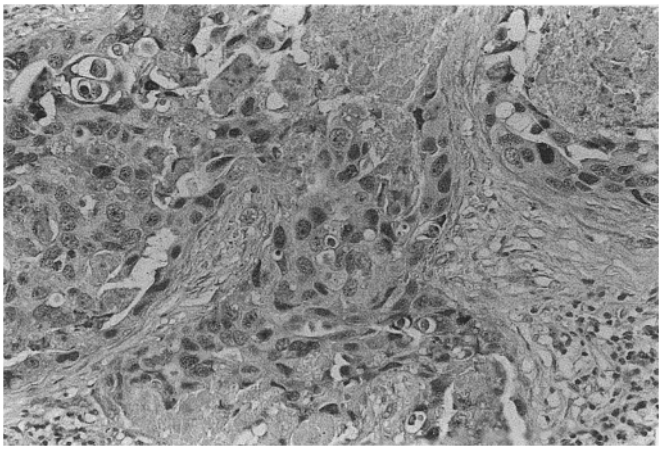

Fig. 4 Invasive ductal carcinoma with a predominant intraductal component showing nuclear staining for p53 (CM1), × 250

Table 2 Comparison between positive and negative cases of p53 protein expression in non-invasive ductal component

	p53 positive (n=18)	p53 negative (n=36)	P value
Histological grade:			
I	0	16	
II	10	17	$P<0.0005$
III	8	3	
Comedo subtypes:			
present	16	10	$P<0.0001$
absent	2	26	

status of c-erbB-2 overexpression (Table 1). Of the 54 samples which contained both intraductal and invasive components, 18 were positive for both components (Figs. 1, 2, 4). On the other hand, 36 samples were negative for both components. Comparing these two groups only with regard to the intraductal components, there was a statistically significant association between p53 protein expression and higher histological grade ($P<0.0005$) or more comedo-subtypes ($P<0.0001$; Table 2). Of the ten samples of IDC with a predominant intraductal component, five with a high histological grade or comedo-subtypes were positive (data not shown) (Fig. 4). Overexpression of c-erbB-2 protein was noted in 16 of 93 cases (17.2%) of IDC including that with a predominant intraductal component. There was a statistically significant association between overexpression of c-erbB-2 protein and higher histological grade ($P<0.01$) or mitotic index ($P<0.05$; data not shown). There was no statistically significant association between overexpression of c-erbB-2 protein and expression of p53 protein. Of the 13 samples of NIDC, only 1 case showed expression of p53 protein and 2 cases showed overexpression of c-erbB-2 protein. No nuclear staining of p53 CM1 was evident in any of the ADH, moderate or florid hyperplasia, or mild hyperplasia.

Discussion

Wild-type p53 immunoreactivity is usually not seen in normal cells because the protein has a very short half-life and does not accumulate to detectable levels (Reihsaus et al. 1990). Mutations of the *p53* gene often result in the production of an abnormal protein with altered conformation and prolonged half-life (Hollstein et al. 1991; Levine et al. 1991). These mutant proteins accumulate in the cell nucleus and can be detected by immunohistochemical staining. Although pitfalls in screening p53 abnormalities by immunohistochemistry must be emphasized (loss of antigenicity during fixation or embedding, gene mutations without protein expression) immunohistochemical analysis is more sensitive than gene analysis (Esrig et al. 1993). We therefore, evaluated p53 abnormalities by immunohistochemistry. The significance of

ADH as a precancerous lesion has not been clarified, but histopathological diagnosis and biological differences between ADH and cancer are very important. Studies of expression of p53 protein in ADH are few (Bartek et al. 1990). We could not detect p53 protein expression in ADH or in benign epithelial hyperplasia in agreement with the results of Bartek et al. (1990). Studies of p53 protein expression or gene mutations in NIDC have also been limited (Bartek et al. 1990; Davidoff et al. 1991; Poller et al. 1993; Tsuda et al. 1993). The largest published study of NIDC (Poller et al. 1993) has shown p53 protein expression in 25.2% of 143 samples, and was confined almost exclusively to large cell NIDC, a morphological subtype of in situ breast carcinoma thought to be biologically aggressive. In our study we revealed only one sample positive, because few NIDC with high histological grade might be included among our samples. IDC with a predominant intraductal component has not been much studied and no gene mutations has been detected (Tsuda et al. 1993). However we found p53 protein expression in five of ten samples. There was a completely coordinated expression of p53 protein in both the intraductal and the invasive component in IDC, including those cases with a predominant intraductal component. The intraductal components in IDC (including those with a predominant intraductal component) which express p53 protein showed a significant correlation with a higher histological grade or mitotic index regardless of status of c-erbB-2 overexpression. These results suggest that expression of p53 protein may also correlate with the early stage of invasion. It has also been suggested that p53 protein expression frequently occurs at the stage of NIDC with a high histological grade or comedo-subtypes and that its expression is maintained during progression from intraductal to invasive carcinoma. Although NIDC and IDC with a predominant intraductal component are commonly thought to have a favourable outcome, patients with p53 protein expression or high histological grade might progress to more invasive stage, resulting in a poor prognosis (Tsuda et al. 1990; Barnes et al. 1993). It is valuable to select these populations by using conventional histological assessment of histological grade and immunohistochemical detection of p53 protein expression.

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