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A mutant *TP53* gene status is associated with a poor prognosis and anthracycline-resistance in breast cancer patients

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Abstract

This study evaluates the prognostic and predictive relevance of a mutated p53 in a series of 254 samples from primary breast cancer patients. C-erbB-2 analysis was defined in a limited subpopulation of 79 patients. p53 and c-erbB-2 status was analysed by immunohistochemical staining of the tumour samples. Positive p53 immunostaining was present in 86 cases (34%) and correlated with a high malignant grade, negative progesterone receptor status and ductal histology of tumour. C-erbB-2 positivity was seen in 38 samples (48%). Within an average follow-up time of 74 months, 121 patients developed recurrent or metastatic disease. Patients with mutated p53 showed a statistically significant shorter overall survival and disease-free survival in both univariate and multivariate analyses. The worst clinical outcome was seen in patients who were both p53- and c-erbB-2-positive. The response rate to anthracycline-based chemotherapy in metastatic disease was low in the p53-positive cases. Our results help to clarify the independent prognostic role of a mutated p53 status in breast cancer patients, indicating that this gene might be predictive of anthracycline resistance. Patients with a mutant p53 status and overexpressing c-erbB-2 should be regarded as high-risk cases.

Keywords: p53; c-erbB-2; Prognosis; Predictive factor; Anthracycline; Breast cancer

1. Introduction

Stage at diagnosis and the biological aggressiveness of a malignant tumour strongly influence the prognosis of a breast cancer patient. Primary tumour size and the nodal status of the axilla are known to have significant prognostic power, but their value in small sized or node-negative cases is limited. In these cases, the grade and the expression of oestrogen and progesterone receptors are more characteristic of the biological phenotype of a tumour and also the need for postsurgical adjuvant therapy. Despite advances in adjuvant treatment, approximately 30% of lymph node-negative patients will die of their cancer [1]. In order to treat patients individually, finding reliable new biological markers especially for early stage breast cancers is of the utmost importance, helping to choose the optimal treatment modalities in different subsets of patients.

The biological marker p53 has emerged as a possible prognostic and predictive factor in breast cancer. *TP53* is a tumour suppressor gene localised to chromosome 17, encoding a 53 kD nuclear phosphoprotein that plays an important role in cell cycle regulation and apoptosis following DNA damage. Somatic *TP53* mutation is a common molecular abnormality found in cancer and is detected in approximately 30% of breast tumours. Mutated *TP53*-positive tumours have been associated with a large primary tumour and a positive nodal status [2] and these patients seem to have shorter disease-free survival [3,6] and poorer outcome [2,7], but whether p53 has any independent prognostic value still remains unclear [4,5].

Whether mutated *TP53* can predict the response to anti-cancer therapy has been the focus of several studies. Two large prospective trials reached opposing conclusions considering a possible resistance to hormonal therapy in metastatic disease [8,9]. There is evidence of

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resistance to anthracycline-based chemotherapy in one large study in the adjuvant setting [10], but this was not found in another large trial [11], or in a few smaller studies [12,13].

c-erbB-2 (HER-2) is an oncogene encoding a 185-kDa epidermal growth factor receptor-like membrane glycoprotein, and amplification of this gene may be associated with independent cellular growth. *HER-2* is amplified and/or overexpressed in approximately 25% of malignant breast tumours, and is more likely to be associated with a node-positive, hormone receptor-negative and large primary tumour status, resulting in an early disease recurrence and poor survival [14]. Current data suggests that amplification of *c-erbB-2* is predictive of resistance to hormonal therapy [15,16], while studies investigating resistance to chemotherapeutics show conflicting results [10,11,17].

This study examines the possible prognostic value of a mutated p53 gene in breast cancer patients in terms of disease-free survival and overall survival. Response to hormonal and chemotherapeutic treatment is evaluated in a subset of metastatic patients in order to evaluate the predictive power of this gene. The effect of co-expression of a mutated p53 and overexpression of c-erb-B2 is also examined.

2. Patients and methods

254 primary breast cancer patients operated upon at the Oulu University hospital from 1982–1998 were included in the study, the majority of patients being treated during 1982–1986. The average age of patients was 56 years (range 25–85 years). The average follow-up time was 74 months (range 3–228 months). 121 patients (48%) developed recurrent or metastatic disease. The patients' characteristics are shown in Table 1. The stage of breast cancer was determined according to the TNM classification from the International Union Against Cancer (UICC). Histopathology was determined according to the World Health Organization (WHO) criteria and malignancy grade using the Elston and Ellis method. 115 patients (45%) were node-negative and 123 (48%) node-positive, the nodal status being unknown in 16 cases. Oestrogen and progesterone receptors were analysed either by using radioimmunoassay or immunohistochemical methods.

Mutated p53 and c-erbB-2 overexpression was examined by immunohistochemistry. A mouse monoclonal antibody against the anti-p53 antibody (Do7) and c-erbB2 (NCL-CB11) were purchased from Novocastra Laboratories (Newcastle upon Tyne, UK). The dilutions for the primary antibody for p53 and c-erbB2 were 1:1000 and 1:500, respectively. The immunostaining was performed using the avidin-biotin peroxidase method and the chromogen used was diaminobenzidine (Dakopatts, Copenhagen, Denmark). Negative control stainings were carried out by substituting phosphate buffered solution (PBS) or non-immune mouse serum for the primary antibodies.

p53 status was analysed for all 254 primary tumours, while c-erbB-2 analyses were available in only a limited subpopulation of 79 patients. Adjuvant chemotherapy was given to 37 (15%) and hormonal therapy to 60 (24%) patients. In the subpopulation with recurrent disease, p53-negative and p53-positive groups were comparable in terms of adjuvant therapies. 18 p53-positive patients and 14 p53-negative patients received adjuvant hormonal treatment. 15 patients had received adjuvant chemotherapy in both the p53-positive and -negative groups. 6 patients with positive c-erbB2 received both cytotoxic and hormonal adjuvant therapies in contrast to those in the c-erbB2-negative group where combination therapy was not used. 9 c-erbB2-positive patients received chemotherapy and 8 patients were treated with hormonal therapy, the number being 6 and 12, respectively, in the c-erbB2-negative patients.

The study protocol was approved by the Ethical Committee at the Faculty of Medicine, University of Oulu and the Oulu University Hospital.

2.1. Statistical analyses

Statistical significance was tested using standard tests: Student *t*-test, Mann–Whitney, Pearson and Chi square. Survival curves (Kaplan–Meier) were compared by using the log rank, Breslow or Tarone–Ware test. $P<0.05$ was considered as statistically significant. Cox regression analysis and stepwise regression analysis were used to find significant predictors of survival. In order to evaluate the prognostic power of mutant p53 together with the Nottingham Prognostic Index (NPI) [18], we created a modified prognostic grouping (NPI) and replaced the size of the primary tumour in the NPI with the T-classification since the exact diameter of the primary tumour in millimetres was not available in all cases.

3. Results

3.1. Mutated p53 and c-erbB-2 overexpression in breast cancer patients

Positive p53 immunostaining was present in 86 cases (34%) and 154 cases remained negative (61%). p53 status could not be determined in 14 cases (6%). Positive c-erbB-2 expression was seen in 38 out of 79 tumours (48%), 41 cases were negative. A statistically significant association was seen between mutant p53 and high malignancy grade ($P=0.001$), negative progesterone receptor status ($P=0.04$) and ductal type of histology ($P=0.05$). Correlation between mutant p53 and positive

Table 1
Positive p53 in relation to other breast tumour characteristics

Factor	Patients *n*	(%)	p53 status	*P* value[a]
Menopausal status				0.8
Pre	104	63	Negative	
		37	Positive	
Post	138	65	Negative	
		35	Positive	
Unknown	12			
Tumour size				0.2
T1	78	72	Negative	
		28	Positive	
T2	114	63	Negative	
		37	Positive	
T3	34	59	Negative	
		41	Positive	
T4	15	47	Negative	
		53	Positive	
Unknown	15			
Node status				0.07
N+	123	57	Negative	
		43	Positive	
N−	115	71	Negative	
		29	Positive	
Unknown	16			
Stage				0.4
I	54	70	Negative	
		30	Positive	
II	127	48	Negative	
		52	Positive	
III	29	52	Negative	
		48	Positive	
IV	28	61	Negative	
		39	Positive	
Unknown	16			
Histology				0.05
Ductal	209	63	Negative	
		37	Positive	
Lobular	15	93	Negative	
		7	Positive	
Other	9		Negative	
Unknown	15			
Malignancy grade				0.001
I	20	85	Negative	
		15	Positive	
II	82	72	Negative	
		28	Positive	
III	91	49	Negative	
		51	Positive	
Unknown	61			
ER status				0.5
ER+	119	67	Negative	
		33	Positive	
ER−	8	63	Negative	
		37	Positive	
Unknown	46			
PR+ status				0.04
PR+	112	71	Negative	
		29	Positive	
PR−	95	58	Negative	
		42	Positive	
Unknown	47			

ER+, oestrogen receptor-positive; ER−, oestrogen receptor-negative; PR+, progesterone receptor-positive; PR−, progesterone receptor-negative; N+, node-positive; N−, node-negative.

[a] Pearson Chi square.

nodal status did not reach statistical significance ($P=0.07$) (Table 1), neither was there any statistical significance between the metastatic sites according to p53 status (data not shown). Bone was the most frequent metastatic site in the entire patient group (41%).

3.2. Mutated p53 and its relationship to survival

The average survival time in the p53-positive group was 99 months (95% CI Confidence Interval 75–123), compared with 159 months (95% CI 135–183) in the p53-negative group ($P=0.0005$) (Fig. 1). 28 patients (11%) with primarily metastatic disease were excluded from the analysis. In a univariate analysis, patients with a positive nodal status ($P=0.0001$), large primary tumour ($P=0.000$), high malignancy grade ($P=0.000$), high clinical stage ($P=0.0009$), negative progesterone receptor ($P=0.001$) and positive p53 staining ($P=0.000$) had an impaired overall survival in the study population (Table 2). In multivariate Cox stepwise regression analysis, mutant p53 ($P=0.001$), positive nodal status ($P=0.002$), high clinical stage ($P=0.006$) and negative progesterone receptor status ($P=0.012$) emerged as independent prognostic factors, while negative oestrogen receptor status, histological grade or primary tumour size failed to reach statistical significance. Poor prognosis correlated with a positive p53 status in node-negative patients too, where the average OS was 146 months (95% CI 115–176) compared with 189 months in the p53-negative cases (95% CI 178–200) ($P=0.000$). Patients with recurrent or metastatic disease in the p53-positive group showed a significantly shorter survival than p53-negative patients ($P=0.0005$) (Fig. 2).

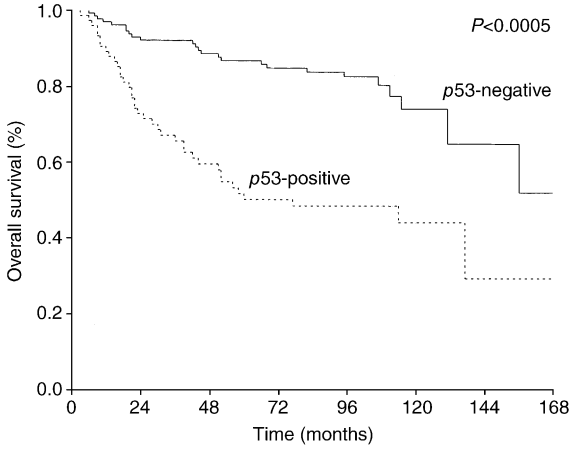

Fig. 1. Overall survival (OS) in the study group in relation to p53 positivity; - - - - = positive ($n=65$); — = negative ($n=142$).

Table 2
Univariate analysis: overall survival (OS) and disease-free survival (DFS)

Prognostic factor	OS patients (n)	OS *P* value [a]	OS relative risk	DFS patients (n)	DFS *P* value [a]	DFS relative risk
Grade						
Grade I	19		1	19		1
Grade II	77	0.052	7.3	78	0.03	3.1
Grade III	79	0.009	14.0	80	0.005	4.4
Tumour size						
T1	74		1	75		1
T2	107	0.0001	2.1	107	0.14	1.4
T3	30	0.022	4.8	30	0.001	2.6
T4	7	0.002	5.5	7	0.000	7.2
Nodal status						
Negative	144		1	115		1
Positive	73	0.0001	6.6	104	0.000	3.2
Stage						
I	55		1	55		1
II	131	0.02	2.5	132	0.111	1.5
III	32	0.0001	10.4	32	0.000	5.8
Receptor status						
ER+	98		1	99		1
ER−	86	0.007	2.0	86	0.147	1.3
PR+	95		1	96		1
PR−	88	0.001	2.4	88	0.087	1.4
p53 mutated						
No	142		1	135		1
Yes	65	0.0001	3.4	74	0.000	2.2

ER+, oestrogen receptor-positive; ER−, oestrogen receptor-negative; PR+, progesterone receptor-positive; PR−, progesterone receptor-negative.
[a] Pearson Chi square.

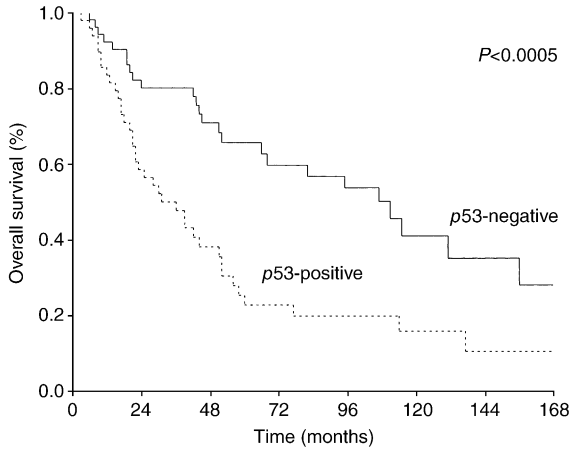

Fig. 2. Overall survival of patients with metastatic disease in relation to p53 positivity, - - - - = metastatic disease, p53-positive ($n=49$); — = metastatic disease, p53-negative ($n=53$).

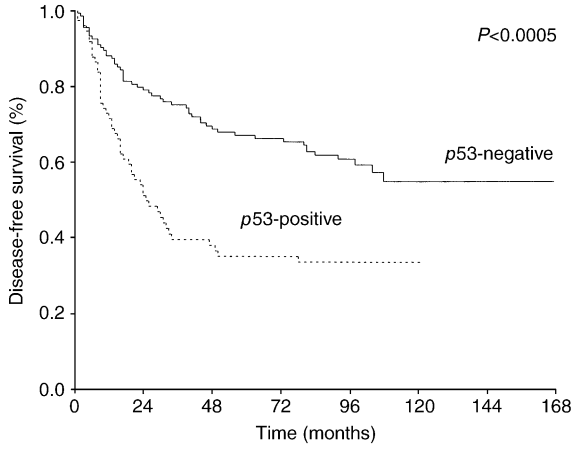

Fig. 3. Disease-free survival (DFS) in the study group in relation to p53 status; - - - - = positive ($n=74$); — = negative ($n=135$).

Mutant p53 status ($P=0.000$), large primary tumour ($P=0.000$), positive nodal status ($P=0.000$) and higher clinical stage ($P=0.000$) emerged as strong prognostic factors in terms of disease-free survival (DFS) (Table 2). Among 121 patients with a recurrence of the disease, there were 53 recurrences in the p53-negative group and 49 recurrences in the p53-positive group (Fig. 3). Median time to disease recurrence was 27 months (range 1–180 months). The prognostic significance of p53 positivity was also confirmed in node-negative patients, where the median DFS was 125 months (95% CI 107–142) in p53-negative cases compared with 53 months (95% CI 42–65) in p53-positive cases, the difference being statistically highly significant ($P=0.0001$). High clinical grade ($P=0.001$), mutant p53 ($P=0.001$) and lymph node metastases of the axilla ($P=0.004$) were independent indicators for a shortened DFS in a multivariate Cox stepwise regression analysis, which included also negative oestrogen receptor status, histological grade and primary tumour size.

The NPI predicted both DFS and OS of patients, but did not overlap with the prognostic power of mutant p53 in a separate Cox regression multivariate analysis (Table 3).

3.3. Co-expression of mutated p53 and amplified c-erbB-2

Expression of both mutated p53 and positive c-erbB-2 in tumours was associated with a poor clinical outcome. The average overall survival was 132 months (95% CI 87–176) in the wild-type p53 and c-erbB-2-negative group, compared with an average OS of only 31 months (95% CI 18–44) in patients with mutated p53 and amplified c-erbB-2 (Fig. 4). Despite the low numbers in these subsets of patients, the result was statistically highly significant ($P=0.0003$). Average survival was 66 months (95% CI 38–93) among patients with p53-positive and c-erbB-2-negative tumours and 74 months (95% CI 45–103) in a subgroup of patients with p53-negative and c-erbB-2-positive tumours.

3.4. Mutated p53 and response to hormonal and chemotherapeutic agents

The responses of 108 patients receiving hormonal or chemotherapeutic treatments for metastatic breast cancer were analysed (Table 4). 13 cases out of a total of 121 recurrences were excluded because of an unknown p53 mutation status. Patients with mutated p53 seemed to be resistant to anthracycline therapy; disease progression was seen in 11 out of 15 treated patients (73%). p53 status did not correlate with the responsive to hormonal agents. Correlation between the response to non-anthracycline based chemotherapy and the p53 status could not be evaluated because of the small number of patients treated. CMF (cyclophosphamide–methotrexate–5-fluorouracil), CEF (cyclophosphamide–epirubicin–5-fluorouracil) and FA (5-fluorouracil-doxorubicin) were the most frequent chemotherapy regimens used in this study.

Table 3
Multivariate analysis: Mutant p53 and the Nottingham Prognostic Index (NPI) as predictors of overall survival (OS) and disease-free survival (DFS)

Prognostic factor	OS and DFS patients (n)	OS P value	DFS P value
P53 mutated	62	0.013	0.055
NPI	166	0.000	0.000

Table 4
Response to chemotherapy and/or hormonal therapy according to p53 status in metastatic breast cancer patients

Treatment	Wild-type p53 response (%)			Mutated p53 response %		
	CR + PR	SD	PD	CR + PR	SD	PD
Anthracycline-based chemotherapy	5/15 (33)	5/15 (33)	5/15 (33)	4/15 (27)	0	11/15 (73)
Non-anthracycline-based chemotherapy	1/7 (14)	2/7 (29)	4/7 (57)	1/5 (20)	0	4/5 (80)
Hormonal therapy	12/37 (32)	9/37 (24)	16/37 (43)	12/30 (40)	3/30 (10)	15/30 (50)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; according to EORTC Breast Cancer Cooperative Group, Manual for Clinical Research in Breast Cancer, 1998; EORTC, European Organization for Research and Treatment of Cancer.

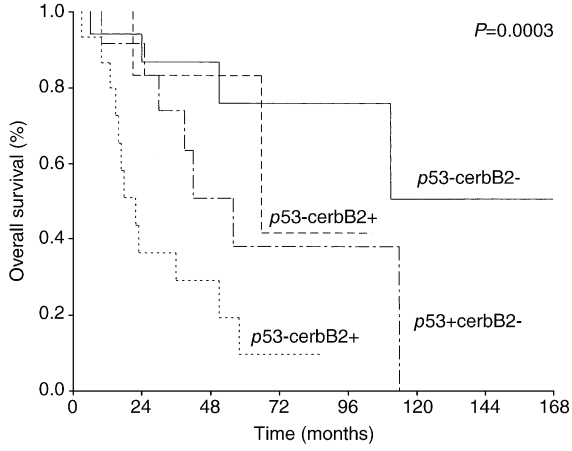

Fig. 4. Overall survival in patients in relation to p53 and c-erb-B-2 status; ……= both p53- and c-erbB-2-positive ($n = 12$); -.-.-.-= p53-positive and c-erbB-2-negative ($n = 7$); - - - -= p53-negative and c-erbB-2-positive ($n = 2$); —= both p53- and c-erbB-2-negative ($n = 4$).

4. Discussion

Our data suggests mutated p53 is an independent prognostic factor in breast cancer patients. p53 status also emerged as a predictor of anthracycline-based chemotherapy resistance. We also demonstrated that co-expression of p53 and c-erbB-2 has an impact on the overall survival of breast cancer patients.

Positive correlation between mutant p53 and classical prognostic factors, such as high malignant grade, positive nodal status and negative progesterone receptor status has also been previously reported [2,4,6]. The prevalence of mutated p53 observed here (34%) is similar to that observed in other studies [5,19]. Mutant p53 correlated here with a poor overall survival, confirming results obtained in several other reports [2,7,20]. Mutant p53 was an independent prognostic factor in this study, contrary to many other reports [4,20]. There are conflicting data with regard to the significance of mutant p53 in node-negative patients [4,5,21], but this connection was highly relevant in this study.

In our study we showed that co-expression of mutated p53 and positive c-erbB-2 dramatically worsened the overall survival of the patients. Few studies have demonstrated this association [22,23], and only one of them has investigated prognosis in such a subgroup of patients as in the present study [23]. Mutated p53 alone has a stronger effect on OS than c-erbB-2 positivity alone. Average OS was more than five times longer in cases with wild-type p53 and c-erbB-2 negativity. The reason for this may be partly related to the poor responsive to chemotherapy in metastatic disease reported here or to aggressive behaviour of the tumours.

30 patients received anthracycline-based chemotherapy due to disease recurrence. Treatment failure was frequently seen in the p53-positive group (73%) compared with the p53-negative group (33%). Muss and colleagues [11] found mutant p53 to be predictive of resistance to anthracycline-based chemotherapy in the adjuvant setting, unlike Clahsen and colleagues [10]. Several reports have been published with small numbers of patients and conflicting results [24]. Because only a small number of patients were treated with non-anthracycline-based chemotherapy, the predictive value of p53 in this set of patients could not be assessed in this treatment modality. Previous conflicting data suggest both resistance and sensitivity to non-anthracycline-based chemotherapy in p53-mutated patients [24]. Previous studies also report conflicting results with regard to the responsive to tamoxifen in metastatic disease cases [8,9]. No major differences were seen in our study in terms of responsive to hormonal therapy according to the p53 status.

The conflicting data may be partly explained by the differing methodology used to assess p53 mutations. Immunohistochemistry, as used here, is an inexpensive and simple method, but results are subjectively interpretated. Hamilton and Piccart [24] proposed that immunohistochemistry gives negative results more often than positive results, which was emphasised in the results we show here. DNA sequencing is an exact method, but in order to get reliable information the complete gene should be sequenced, and different mutation sites affect clinical relevance [21]; this laborious method seems to be more appropriate for experimental than for clinical studies.

The *TP53* tumour suppressor gene plays a major role in the stress-mediated cell cycle, but this mechanism may be modulated by the use of taxanes. There are

preliminary results of responses to taxane treatment in ovarian cancer patients with a mutant p53 status [25], and further studies should be carried out to determine the predictive power of this gene when treating breast cancer patients with new anticancer drugs. In the future, p53 will probably emerge not only as a predictive factor, but also as a target for drugs restoring the altered p53 regulatory mechanism in malignant cells (ASCO abstr 50, 2001).

The data demonstrated here, and in other studies, could encourage clinicians to use new biomarkers when evaluating the individual risk of recurrence in breast cancer patients. Particularly patients with a mutant p53 and c-erbB-2 positive status seem to have a poorer prognosis and might benefit from well conceived adjuvant and therapies once the disease recurs.

Acknowledgements

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