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CLINICAL TRIAL

Prognostic effects of abnormal DNA damage response protein expression in breast cancer

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Abstract

Purpose We aimed to explore the expression of DNA damage response machinery proteins and their integrated prognostic value in different subgroups of breast cancer.

Methods Expression of NBS1, BRCA1, BRCA2, ATM, and p53 was determined by immunohistochemistry in 419 surgically resected breast tumors.

Results Loss of NBS1, BRCA1, ATM, and abnormal p53 expression was significantly associated with lower disease-free survival rates. Abnormal DNA damage response protein expression, defined as loss of any one of NBS1, BRCA1, ATM, and/or abnormal p53 expression, was observed in 258 of 399 evaluable cases (64.7%) and was significantly associated with higher tumor grade, larger tumor size, and ER-negative, and/or PR-negative status. Most patients with luminal B (86.1%), HER2-enriched (94.4%), and triple-negative (86.8%) breast cancers had abnormal DNA damage response protein expression. In contrast, abnormal DNA damage response protein expression was found in only 53.8% of luminal A tumors. Abnormal DNA damage response protein expression was associated with significantly lower 5-year disease-free survival rates in all patients (95.6% vs. 84.8%, $p=0.001$), as well as in the luminal A subgroup (97.4% vs. 89.0%, $p=0.011$). In multivariate analysis, abnormal DNA damage response protein expression remained an independent predictor of shorter disease-free survival for luminal A subtype (hazard ratio 3.14, 95% confidence interval 1.16–8.47; $p=0.024$).

Conclusion Abnormal DNA damage response protein expression is found in most luminal B and HER2-enriched breast cancers as frequently as in triple-negative breast cancer. In the luminal A subtype, abnormal DNA damage response protein expression is an independent prognostic marker.

Keywords DNA damage · DNA repair · Homologous recombination · BRCA1 protein · BRCA2 protein · Prognosis

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Introduction

DNA damage repair is a new area for anti-cancer drug discovery in breast cancer. Among multiple DNA repair mechanisms, homologous recombination is a robust, error-free mechanism for DNA double-strand break repair, and multiple components participate in the homologous recombination repair process. The Mre11–Rad50–Nbs1 complex, composed of meiotic recombination 11 homolog (MRE11), RAD50, and Nijmegen breakage syndrome 1 (NBS1), detects DNA double-strand breaks and activates the ataxia-telangiectasia mutated (ATM) kinase, which is a master regulator of DNA damage response [1]. ATM phosphorylates downstream targets, such as CHK1, act to reduce cyclin-dependent kinase activity, which in turn slows down or arrests cell-cycle progression to increase the time available for DNA repair [2]. BRCA1/2 also mediate rate-limiting events in homologous recombination, and loss of p53 function cooperates with the loss of BRCA1/2 in tumorigenesis [3–5]; chromosome breaks caused by loss of BRCA1/2 function activate p53-dependent checkpoint controls and/or apoptosis to prevent tumor formation.

Tumors with homologous recombination defects, such as BRCA1 and BRCA2 mutated breast cancers, are not able to repair double-strand breaks induced by poly (ADP-ribose) polymerase (PARP) inhibitors, and are therefore sensitive to PARP inhibitors, as well as DNA damaging chemotherapeutics. A recent study suggested that homologous recombination defects are also associated with the efficacy of immune checkpoint inhibitor treatment [6]. In this regard, finding tumors with homologous recombination defects is of importance in identifying the appropriate target population for these treatment approaches. However, there is currently no standard method to identify tumors with homologous recombination defects. Microarray-based analysis of 44 genes and their homologous recombination deficiency scores, defined as the number of loss of heterozygosity regions, have been suggested [7, 8]; however, these assays are not readily available in daily clinical practice.

In this study, we aimed to understand the immunohistochemical expression of key proteins in the DNA damage response machinery involved in breast cancer, and to investigate whether the integrated expression pattern can identify a subset of breast cancers with different clinicopathological characteristics and prognosis.

Patients and methods

Study population

A total of 419 consecutive breast cancer patients who underwent curative-intent resection in 2008 at Seoul National University Hospital, Seoul, Korea, were enrolled in the study. Clinical data were retrieved from patient medical records. The median follow-up period was 89.7 months (range 3.6–101.9 months). Primary treatments included radical mastectomy, modified radical mastectomy, and breast-conserving surgery, as well as sentinel lymph node biopsy or axillary lymph node dissection. Adjuvant chemotherapy and radiotherapy were administered at the discretion of the physician. The Institutional Review Board at Seoul National University Hospital approved this study (H-1409-017-607), and it was conducted in accordance with Declaration of Helsinki ethical principles. Written informed consent was not required because it was a retrospective study, and all patients' records/information were anonymized and deidentified prior to analysis.

Immunohistochemical analysis of DNA damage response proteins

Tumor microarray construction was completed as previously described [9]. The primary antibodies were diluted as follows: estrogen receptors (ER) (1D5; Novocastra Laboratories, Newcastle, UK), 1:100; progesterone receptors (PR) (PgR636; DAKO, Hamburg, Germany), 1:200; p53 (DO-7; DAKO, Hamburg, Germany), 1:500; ATM (ab32520; Abcam, UK), 1:100; NBS1 (NB100-143; Novus), 1:1200; BRCA1 (OP92; Calbiochem, Darmstadt, Germany), 1:100; and BRCA2 (MAB2476; R&D systems), 1:500. Nuclear expression of tumor cells was interpreted as positive for ER, PR, ATM, NBS1, BRCA1/2, and p53, while membrane staining of tumor cells was considered positive for HER2. Immunohistochemical staining was evaluated on the basis of the location and percentage or intensity of positively stained cells. Immunohistochemical scoring was performed with investigators blinded to all other patient data, including outcomes. To ensure accuracy, two experienced breast pathologists (H.S.R. and H. K.) reviewed all of the immunohistochemical stainings without knowledge of patient outcomes.

Immunohistochemical staining for ER and PR expression was counted and categorized as positive when $\geq 1\%$ of the tumor cells were stained according to the 2010 American Society of Clinical Oncology/College of American Pathologists guidelines [10]. Immunohistochemical expression of HER2 was assessed based on

the 2013 American Society of Clinical Oncology/College of American Pathologists guidelines [11]. We classified breast cancer patients into four subgroups, namely luminal A, luminal B, HER2-enriched, and triple negative breast cancer according to the 2011 St. Gallen Consensus Panel [12]. Loss of NBS1 was defined as no detectable staining in tumor cells. Positive staining of tumor cells with any intensity was considered positive for BRCA1 and BRCA2. Loss of ATM was defined as < 10% of cells with weakly positive (+/3) or higher intensity, that is, > 90% of cells showing negative (0) or equivocal staining (±), as previously described [9, 13]. Abnormal p53 expression was defined as either positive labeling in 50% or more of tumor cell nuclei or total absence of nuclear labeling, as previously described [14–16].

Statistical analysis

The baseline characteristics of patients and clinicopathological findings according to DNA damage response protein expression were evaluated using the Pearson Chi-square or Fisher exact test for categorical variables. The period of disease-free survival was defined as the duration of time between the date of surgical resection and one of the following events: disease relapse, any cause of death before disease relapse, or the last follow-up. Disease-free survival was calculated using the Kaplan–Meier method, and values were compared using the log-rank test. Univariate Cox proportional-hazard regression (PHR) analyses were performed to evaluate the predictive values of each variable, and variables found to be significant on univariate analysis were introduced into a multi-variable Cox PHR model for disease-free survival. All tests were two-sided, and a p value of less than 0.05 was indicative of a statistically significant difference. All analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA)and SPSS version 21.0 (SPSS; Chicago, IL), on data collected through July 2016.

Results

Expression of NBS1, BRCA1, BRCA2, ATM, and p53 and patient outcome

All 419 patients were female, with a median age of 47 years. Of the 419 patients, 148 (34.3%), 231 (53.6%), and 40 (9.3%) had stage I, II, and III disease, respectively. Three hundred and twenty-five (75.4%) patients were treated with adjuvant chemotherapy, 275 (63.8%) with radiotherapy, and 300 (69.6%) with hormone therapy. NBS1 and BRCA1 protein expression patterns are shown in Fig. 1. The associations of NBS1, BRCA1, and BRCA2 expression with clinicopathological characteristics are detailed in Table 1.

NBS1

Loss of NBS1 was observed in 27 out of 406 evaluable tumors (6.7%) and was significantly associated with higher nuclear grade (9.5% vs. 2.9%, $p = 0.008$), histologic grade (10.0% vs. 2.7%, $p = 0.003$), larger tumor size (10.0% in T3 vs. 10.6% in T2 vs. 2.6% in T1, $p = 0.015$), negative ER expression (13.6% vs. 4.1%, $p = 0.001$), negative PR expression (13.2% vs. 3.6%, $p < 0.001$), and high Ki-67 index (≥ 14%) (14.6% vs. 5.6%, $p = 0.029$). With respect to molecular breast cancer subtypes, NBS1 loss was less frequently observed in luminal A breast cancers than in the other subtypes (3.7% in luminal A, 8.3% in luminal B, 10.8% in HER2 enriched, and 18.9% in triple negative breast cancer subtypes; $p = 0.001$). Univariate analysis revealed that tumoral NBS1 loss was significantly associated with lower disease-free survival rates than intact NBS1 (5-year disease-free survival rate of 73.9% vs. 90.4%, HR 2.56; 95% CI 1.15–5.70; $p = 0.017$) (Fig. 2a).

BRCA1

BRCA1 loss was observed in 33.7% (137 out of 404 evaluable tumors) and was significantly associated with higher nuclear grade (39.3% vs.26.9%, $p = 0.009$), histologic grade (39.5% vs.27.2%, $p = 0.009$), and presence of LN metastasis (40.1% vs. 30.0%, $p = 0.035$). BRCA1 loss was also associated with lower disease-free survival than intact BRCA1 (5-year disease-free survival rate of 84.9% vs. 91.4%; HR 1.98, 95% CI 1.15–3.43, $p = 0.013$) (Fig. 2b).

ATM and p53

ATM and p53 expression and their association with clinicopathological parameters have been previously reported [9]. Briefly, ATM loss was observed in 30.8% of patients and was associated with higher T stage (40% in T3 vs. 37.2% in T2 vs. 24.4% in T1, $p = 0.030$), presence of LN metastasis (38.6% vs. 26.1%, $p = 0.006$), and negative ER (43.1% vs. 26.5%, $p = 0.001$) and negative PR (44.2% vs. 24.8%, $p < 0.001$) expression. Abnormal p53 expression was observed in 39.3% of patients and was associated with higher T stage (40% in T3 vs. 46.2% in T2 vs. 32.3% in T1, $p = 0.040$), ER-negative (77.6% vs. 25.3%, $p < 0.001$) and/or PR-negative (69.0% vs. 25.1%, $p < 0.001$) status, positive HER2 status (72.1% vs. 33.7%, $p < 0.001$). ATM loss was associated with a significantly lower disease-free survival rate than intact ATM (5-year disease-free survival rate of 83.5% vs. 91.8%; HR 1.84, 95% CI 1.05–3.19, $p = 0.029$), and abnormal p53 expression was also associated with

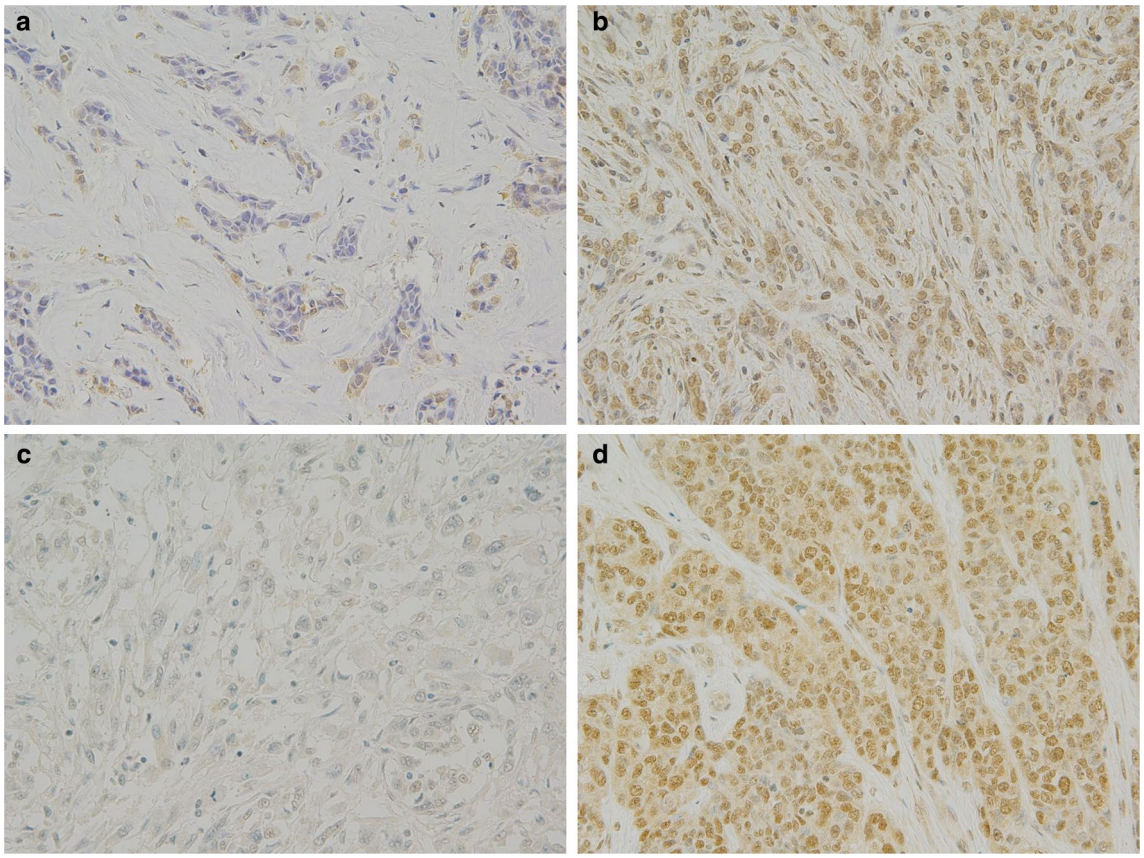

Fig. 1 Immunohistochemical expression of BRCA1 and NBS1 proteins in breast cancer. **a** Loss of BRCA1 expression, **b** intact BRCA1 expression; **c** loss of NBS1 expression; **d** positive NBS1 expression (magnifications, ×400)

poorer outcome (5-year disease-free survival rate of 81.7% vs. 93.7%; HR 2.46, 95% CI 1.41–4.31, $p=0.002$).

BRCA2

Positive expression of BRCA2 was observed in 41 out of 407 evaluable cases (10.1%) and was not significantly associated with any clinicopathological parameters assessed (Table 1). Furthermore, BRCA2 expression was not significantly associated with survival (5-year disease-free survival rate of 92.3% for positive BRCA2 expression vs. 88.9% for negative BRCA2 expression; HR 1.37, 95% CI 0.49–3.79, $p=0.548$). Correlation between DNA damage response protein expression is shown in Table 2.

Abnormal DNA damage response protein expression and patient outcome

Normal DNA damage response protein expression was defined as intact NBS1, BRCA1, and ATM expression, as well as normal p53 expression. We did not incorporate BRCA2 into the model since BRCA2 expression was not associated with clinicopathological variables, as well as clinical outcomes. All other tumors with abnormal expression of any one of the DNA damage response proteins were considered to have abnormal DNA damage response protein expression.

Abnormal DNA damage response protein expression was observed in 258 of 399 evaluable cases (64.7%), and the associations of DNA damage response protein expression with clinicopathological characteristics are detailed in Table 3. Most of the tumors with luminal B, HER2-enriched and triple-negative subtypes had abnormal DNA damage response protein expression (86.1%, 94.4%, and 86.8%, respectively). In the luminal A subtype, 53.8% (142/264) of the patients had abnormal DNA damage response protein expression.

Abnormal DNA damage response protein expression was significantly associated with a lower disease-free survival rate than normal DNA damage response protein expression (5-year disease-free survival rate 95.6% vs. 84.8%; HR 3.31, 95% CI 1.56–7.05, $p=0.001$) (Fig. 2c). Other clinicopathological factors associated with poor disease-free survival included older age at diagnosis (HR 2.05; 95% CI 1.07–3.89; $p=0.030$), higher T stage (HR 3.09; 95% CI 1.65–5.78; $p<0.001$), presence of lymph node metastasis (HR 2.02; 95% CI 1.17–3.50;

Table 1 Association between NBS1, BRCA1, and BRCA2 expression status and clinicopathological characteristics

Variables	NBS1			BRCA1			BRCA2		
	Loss	Intact	*p* value	Loss	Intact	*p* value	Positive	Negative	*p* value
Age									
< 60	23 (6.6)	326 (93.4)	0.904	121 (34.9)	226 (65.1)	0.315	36 (10.3)	314 (89.7)	0.725
≥ 60	4 (7.0)	53 (93.0)		16 (28.1)	41 (71.9)		5 (8.8)	52 (91.2)	
NG									
Low	5 (2.9)	170 (97.1)	0.008	47 (26.9)	128 (73.1)	0.009	21 (11.9)	155 (88.1)	0.277
High	22 (9.5)	209 (90.5)		90 (39.3)	139 (60.7)		20 (8.7)	211 (91.3)	
HG									
Low	5 (2.7)	180 (97.3)	0.003	50 (27.2)	134 (72.8)	0.009	23 (12.4)	163 (87.6)	0.159
High	22 (10.0)	199 (90.0)		87 (39.5)	133 (60.5)		18 (8.1)	203 (91.9)	
LVI									
Negative	15 (6.0)	233 (94.0)	0.542	81 (32.9)	165 (67.1)	0.602	26 (10.5)	222 (89.5)	0.731
Present	12 (7.6)	146 (92.4)		56 (35.4)	102 (64.6)		15 (9.4)	144 (90.6)	
T									
T1	5 (2.6)	191 (97.4)	0.015	57 (29.1)	139 (70.9)	0.201	23 (11.6)	175 (88.4)	0.580
T2	21 (10.6)	178 (89.4)		76 (38.6)	121 (61.4)		18 (9.1)	180 (90.9)	
T3	1 (10.0)	9 (90.0)		4 (40.0)	6 (60.0)		0	10 (100.0)	
T4	0	1 (100.0)		0	1 (100.0)		0	1 (100.0)	
N									
Negative	13 (5.2)	236 (94.8)	0.145	74 (30.0)	173 (70.0)	0.035	26 (10.4)	223 (89.6)	0.757
Positive	14 (8.9)	143 (91.1)		63 (40.1)	94 (59.9)		15 (9.5)	143 (90.5)	
ER									
Negative	15 (13.6)	95 (86.4)	0.001	44 (40.4)	65 (59.6)	0.096	11 (10.0)	99 (90.0)	0.976
Positive	12 (4.1)	284 (95.9)		93 (31.5)	202 (68.5)		30 (10.1)	267 (89.9)	
PR									
Negative	17 (13.2)	112 (86.8)	< 0.001	52 (40.3)	77 (59.7)	0.063	10 (7.7)	120 (92.3)	0.274
Positive	10 (3.6)	267 (96.4)		85 (30.9)	190 (69.1)		31 (11.2)	246 (88.8)	
HER2									
Negative	21 (6.3)	313 (93.7)	0.407	108 (32.5)	224 (67.4)	0.151	32 (9.6)	303 (90.4)	0.673
Positive	6 (9.7)	56 (90.3)		26 (41.9)	36 (58.1)		7 (11.3)	55 (88.7)	
Ki-67									
<14%	20 (5.6)	338 (94.4)	0.029	121 (34.0)	235 (66.0)	0.928	36 (10.0)	323 (90.0)	0.933
≥14%	7 (14.6)	41 (85.4)		16 (33.0)	32 (66.7)		5 (10.4)	43 (89.6)	
Subtype									
Luminal A	10 (3.7)	260 (96.3)	0.001	83 (31.0)	185 (69.0)	0.319	29 (10.7)	242 (89.3)	0.461
Luminal B	3 (8.3)	33 (91.7)		14 (38.9)	22 (61.1)		2 (5.6)	34 (94.4)	
HER2E	4 (10.8)	33 (89.2)		15 (40.5)	22 (59.5)		5 (13.5)	32 (86.5)	
TNBC	10 (18.9)	43 (81.1)		22 (41.5)	31 (58.5)		3 (5.7)	50 (94.3)	

NG nuclear grade, *HG* histologic grade, *LVI* lymphovascular invasion, *T* tumor size, *N* lymph node involvement, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2

$p = 0.012$), negative expression of ER (HR 2.26; 95% CI 1.30–3.91; $p = 0.004$), and negative expression of PR (HR 2.32; 95% CI 1.35–4.00; $p = 0.002$). Breast cancer subtype also had a prognostic impact; the 5-year disease-free survival rate was 93.7% for luminal A, 74.3% for luminal B, 88.8% for HER2-enriched, and 73.1% for triple-negative subtypes ($p < 0.001$). In multivariate analysis, a tendency toward poorer prognosis was observed for abnormal DNA damage response protein expression, although results were not statistically significant by a narrow margin (HR 2.11, 95% CI 0.96–4.67, $p = 0.064$) (Table 4).

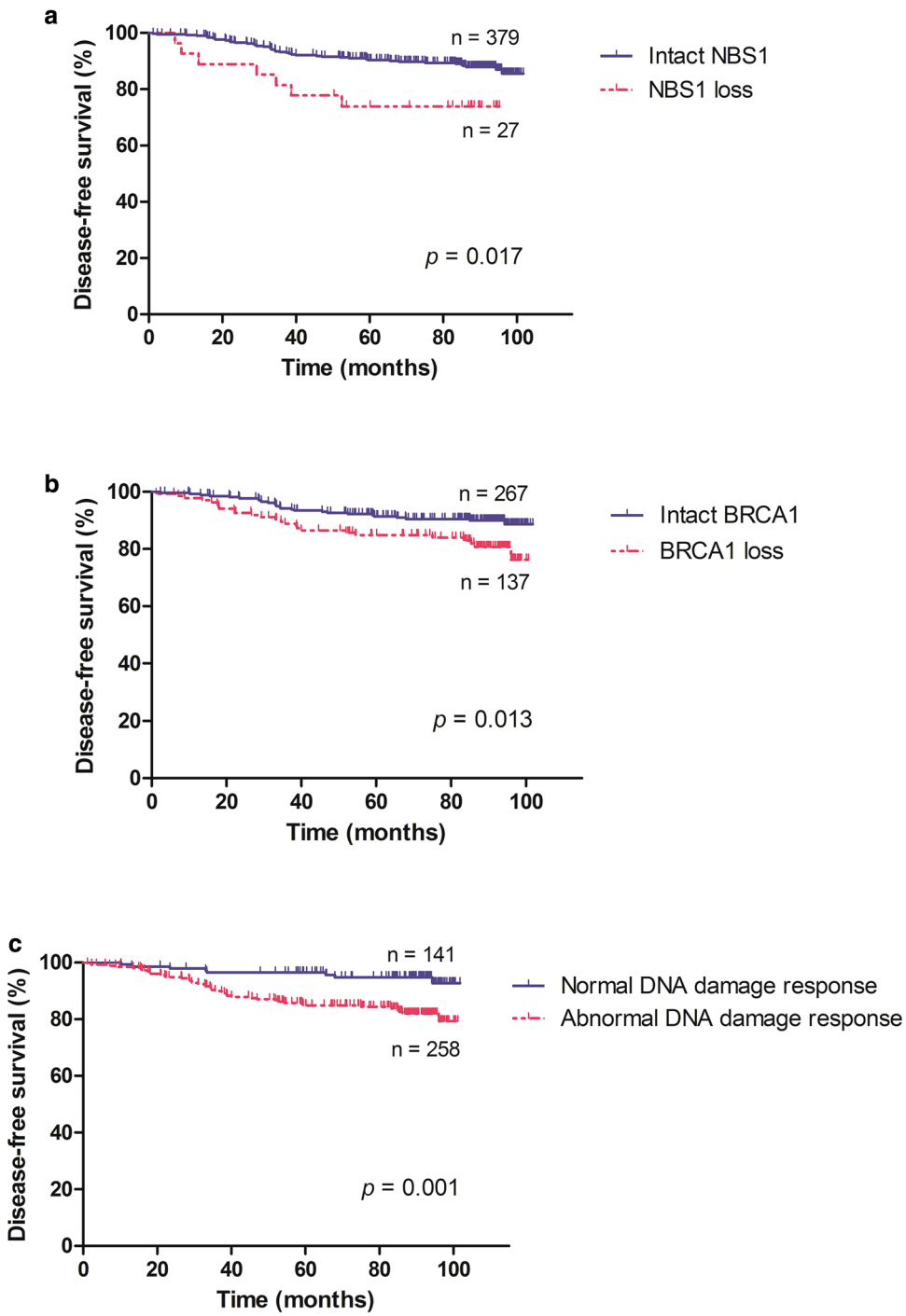

Fig. 2 Disease-free survival according to **a** NBS1 expression, **b** BRCA1 expression, and **c** DNA damage response protein expression

Prognostic significance of abnormal DNA damage response protein expression in luminal A breast cancer subtype

Since most of the tumors with luminal B, HER2-enriched and triple-negative subtypes that had abnormal DNA damage response protein expression status did not have an impact on survival (Fig. 3), we focused on the prognostic impact of DNA damage response protein expression in luminal A subtype tumors.

Among 280 patients with the luminal A subtype, abnormal DNA damage response protein expression was observed in 142 out of 264 evaluable tumors (53.8%). Clinicopathological characteristics of luminal A tumors with abnormal DNA damage response protein expression were similar to those of the overall population (Supplementary Table 1).

Table 2 Correlation between DNA damage response protein expressions

Variables	NBS1			BRCA1			ATM			p53		
	Abnormal	Normal	*p* value	Loss	Intact	*p* value	Loss	Intact	*p* value	Abnormal	Normal	*P* value
BRCA1												
Loss	21 (15.3)	116 (84.7)	< 0.001									
Intact	6 (2.3)	260 (97.7)										
ATM												
Loss	26 (20.8)	99 (79.2)	< 0.001	69 (55.2)	56 (44.8)	< 0.001						
Intact	1 (0.4)	279 (99.6)		68 (24.5)	210 (75.5)							
P53												
Abnormal	23 (14.6)	134 (85.4)	< 0.001	63 (40.1)	94 (59.9)	0.040	65 (41.4)	92 (58.6)	< 0.001			
Normal	4 (1.7)	237 (98.3)		73 (30.2)	169 (69.8)		60 (24.8)	182 (75.2)				
BRCA2												
Positive	2 (4.9)	39 (95.1)	1.000	13 (31.7)	28 (68.3)	0.753	8 (19.5)	33 (80.5)	0.097	10 (25.6)	29 (74.4)	0.065
Negative	25 (6.9)	339 (93.1)		124 (34.2)	239 (65.8)		117 (32.1)	247 (67.9)		147 (40.8)	213 (59.2)	

Table 3 Clinicopathological features of breast cancer patients with normal and abnormal DNA damage response protein expression

Variables	*N*	DNA damage response protein, N (%)		*p* value
		Abnormal	Normal	
Age				
< 60	343	220 (64.1)	123 (35.9)	0.590
≥ 60	56	38 (67.9)	18 (32.1)	
NG				
Low	172	81 (47.1)	91 (52.9)	< 0.001
High	227	177 (78.0)	50 (22.0)	
HG				
Low	181	83 (45.9)	98 (54.1)	< 0.001
High	218	175 (80.3)	43 (19.7)	
LVI				
Negative	241	154 (63.9)	87 (36.1)	0.694
Present	158	104 (65.8)	54 (34.2)	
T				
T1	191	109 (57.1)	82 (42.9)	0.009
T2	197	142 (72.1)	55 (27.9)	
T3	10	7 (70.0)	3 (30.0)	
T4	1	0	1 (100.0)	
N				
Negative	243	150 (61.7)	93 (38.3)	0.126
Positive	156	108 (69.2)	48 (30.8)	
ER				
Negative	108	96 (88.9)	12 (11.1)	< 0.001
Positive	291	162 (55.7)	129 (44.3)	
PR				
Negative	128	106 (82.8)	22 (17.2)	< 0.001
Positive	271	152 (56.1)	119 (43.9)	
HER2				
Negative	328	198 (60.4)	130 (39.6)	< 0.001
Positive	61	56 (91.8)	5 (8.2)	
Ki-67				
< 14%	351	215 (61.3)	136 (38.7)	< 0.001
≥ 14%	48	43 (89.6)	5 (10.4)	
Subtype				
Luminal A	264	142 (53.9)	122 (46.2)	< 0.001
Luminal B	36	31 (86.1)	5 (13.9)	
HER2E	36	34 (94.4)	2 (5.6)	
TNBC	53	47 (88.7)	6 (11.3)	

NG nuclear grade, *HG* histologic grade, *LVI* lymphovascular invasion, *T* tumor size, *N* lymph node involvement, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2

The 5-year disease-free survival rate was significantly lower in the abnormal DNA damage response protein expression group compared to the normal DNA damage response protein expression group (89.0% *vs*. 97.4%, respectively; HR 3.37, 95% CI 1.25–9.07, $p = 0.011$) (Fig. 3). In multivariate

Table 4 Significant independent predictors of disease-free survival on multivariate analysis

Variables	HR (95% CI)	*p* value
DNA damage response protein expression		
Normal	1	
Abnormal	2.11 (0.96–4.67)	0.064
Age		
< 60	1	
≥ 60	2.24 (1.17–4.29)	0.015
N		
Negative	1	
Positive	2.04 (1.15–3.61)	0.015
Intrinsic subtype		
Luminal A	1	
Luminal B	4.04 (1.91–8.55)	< 0.001
HER2-enriched	1.13 (0.38–3.38)	0.829
Triple-negative	3.05 (1.52–6.12)	0.002

Factors significant in the univariate model and included in multivariate analysis were patient age, tumor size, presence of lymph node metastasis, intrinsic breast cancer subtype, and DNA damage response protein expression status

HR hazard ratio, *CI* confidence interval, *HER2* human epidermal growth factor receptor 2

analysis, which included age, tumor size, presence of LN metastases as co-variables, abnormal DNA damage response protein expression remained an independent predictor of poorer disease-free survival (HR 3.14; 95% CI 1.16–8.47, $p = 0.024$) (Table 5).

Discussion

In this study, we found that 64.7% of breast cancer patients who underwent resection had abnormal DNA damage response protein expression defined as loss of any one of NBS1, BRCA1, ATM and/or abnormal p53 expression, and that abnormal DNA damage response protein expression was associated with significantly poorer disease-free survival. We also found differential DNA damage response protein expression according to breast cancer subtype. The majority of patients with luminal B (86.1%), HER2-enriched (94.4%), and triple-negative breast cancer subtypes (86.8%) showed abnormal DNA damage response protein expression. Fewer, but still a significant proportion of patients with the luminal A subtype also had abnormal DNA damage response protein expression (53.8%), and in this subgroup, abnormal DNA damage response expression was an independent prognostic marker for worse disease-free survival.

Some sporadic breast cancers, in addition to BRCA1/2 mutated cancers, are known to possess homologous recombination defects due to mutations or epigenetic inactivation of homologous recombination components. For example, somatic BRCA1 mutations are rarely observed in sporadic breast cancers; however, both BRCA1 mRNA and protein

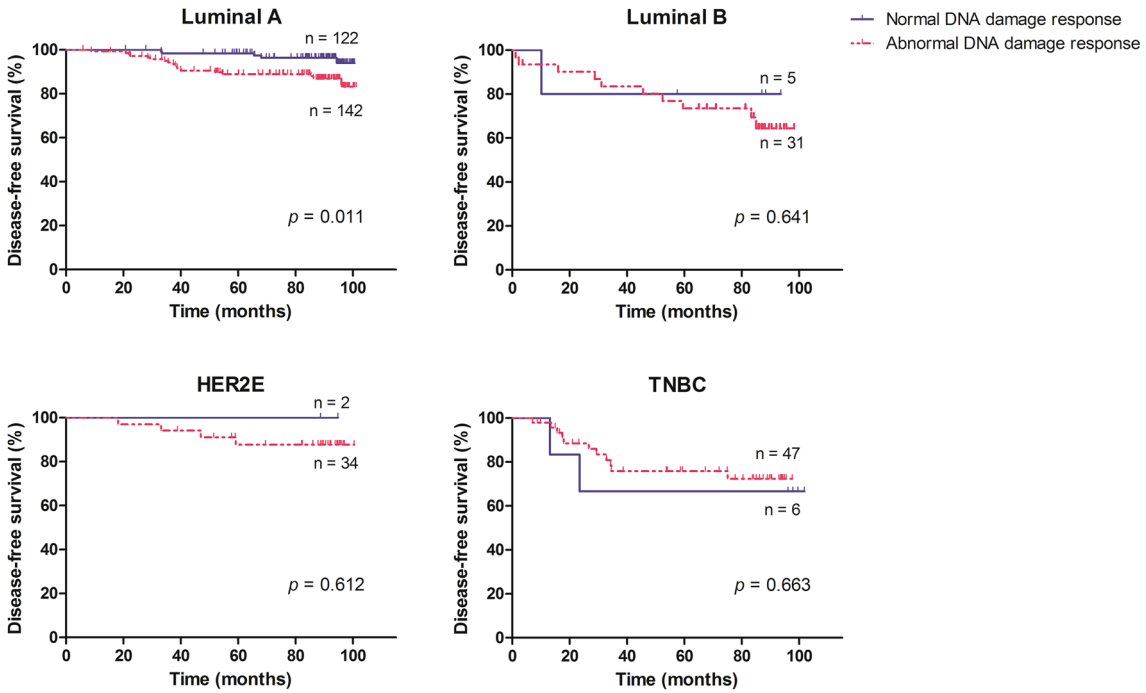

Fig. 3 Disease-free survival according to DNA damage response protein expression in different breast cancer subtypes

Table 5 Significant independent predictors of disease-free survival on multivariate analysis for luminal A subtype patients

Variables	HR (95% CI)	*p* value
DNA damage response protein expression		
Normal	1	
Abnormal	3.14 (1.16–8.47)	0.024
Age		
< 60	1	
≥ 60	3.94 (1.66–9.36)	0.002

HR hazard ratio, *CI* confidence interval

expressions are down-regulated in approximately 30% of sporadic breast cancers [17, 18], possibly due to acquired methylation of the BRCA1 promoter or malfunctions in the upstream pathways that regulate BRCA1 expression. Defining tumors with homologous recombination defects has become an important issue because homologous recombination defects are associated with the efficacy of DNA damaging chemotherapeutics with PARP inhibitors, as well as immunotherapy. In a recent phase II trial of gemcitabine, carboplatin, and iniparib, the homologous recombination deficiency-loss of heterozygosity score was significantly associated with a favorable response to this neoadjuvant platinum and PARP inhibitor treatment [7]. Tumors with high somatic mutational burden are candidates for immunotherapy with agents such as PD-1/PD-L1 checkpoint inhibitors, and in lung cancer, the efficacy of a PD-1 blockade was associated with higher neoantigen burden and DNA repair pathway mutations [6].

In this regard, a number of studies have been devoted to finding a subset of breast tumors with defects in DNA damage response by immunohistochemical analysis of each protein. Several studies, including a previous one by our group, showed that loss of ATM expression was observed in a subset of breast cancers, and this was associated with worse survival [9, 19, 20]. Reduced expression of NBS1 was observed in 10% of breast cancer patients and was significantly associated with shorter survival ($p = 0.0002$) [21]. In terms of BRCA1 expression, M. Aleskandarany et al. reported that, although loss of BRCA1 was more frequently seen in patients with BRCA1 and BRCA2 mutated cancers (76.2% and 95.7%, respectively), a significant proportion of patients with sporadic cancer also had loss of BRCA1 expression (43.8%) [22]. Collectively, expression patterns of each DNA damage response protein in their study were similar to what we observed. Nevertheless, proteins involved in DNA damage response operate interdependently within complex machinery, as described above, and assessing the expression and prognostic value of a single DNA damage response protein might not represent the intactness of the complete DNA damage response machinery properly. To the best of our knowledge, no studies have been devoted to defining the prognostic impact of integrated DNA damage response protein expression in different subtypes of breast cancers. We focused on proteins involved in the homologous recombination pathway, and by simple immunohistochemical analysis of four proteins, we were able to identify subsets of breast tumors with DNA damage response defects that were associated with poor prognosis.

Many studies on breast cancer with DNA damage response defects have focused on the triple-negative subtype because of its similarity to the histologic phenotype and gene expression profiles with BRCA mutated tumors and basal-like (mostly triple-negative) tumors [17, 23]. However, abnormality in homologous recombination is not solely related to basal-like phenotypes, and in the absence of a robust predictive marker of anthracycline-based chemotherapy, as well as PARP inhibitor and immune checkpoint inhibitor treatments, patient selection based only on hormone receptor and HER2 status might result in exclusion of patients who could otherwise benefit from these treatments. Mulligan et al. made a similar effort to ours to identify tumors with DNA damage response defects. In their study, a 44-gene microarray-based assay (the DNA damage response deficiency assay) identified a molecular subgroup of breast cancers deficient in the fanconi anemia (FA)/BRCA DNA damage repair pathway and sensitive to anthracycline/cyclophosphamide-based chemotherapy [8]. This subgroup of DNA damage repair deficient tumors had constitutive activation of the innate immune pathway and was significantly associated with CD4+and CD8+T-cell infiltration and PD-L1 expression [24]. They found that DNA damage repair deficiency predicted outcomes in the neoadjuvant and adjuvant settings independent of ER status, indicating that triple negativity is distinct from DNA damage repair deficiency. In line with their findings, we also found that a subset of luminal A tumors had DNA damage response abnormality, which was an independent prognostic factor for shorter disease-free survival in this patient subgroup. Furthermore, we demonstrated that most of the luminal B and HER2 positive tumors possessed DNA damage response abnormality, which has frequently been observed in the triple-negative subtype. This observation is in line with a previous study which found that inhibitors of Ataxia telangiectasia and Rad3-related proteins, which are sensors of DNA damage and induce homologous recombination-dependent repair, have anti-tumor activity in HER2-positive breast cancer cell lines [25].

Another important finding in our study was that patients with normal DNA damage response expression had extremely good prognosis. Among 141 patients with normal DNA damage response expression, only 8 patients (0.5%) experienced breast cancer recurrence, and none of them had died by the time of analysis. DNA damage response

expression may play a role in screening for breast cancer patients who do not need adjuvant chemotherapy, although this needs to be validated in further studies.

Our study has some limitations. It was a retrospective analysis, and although a relatively large number of patients were included, further studies in additional populations are needed to confirm the findings.

In summary, we demonstrated the clinical significance of integrated DNA damage response protein expression in breast cancer. Abnormal DNA damage response protein expression is seen in luminal B and HER2-enriched subtypes, as frequently as in the triple-negative subtype. In addition, > 50% of luminal A subtype patients also had abnormal DNA damage response protein expression, which has been proven to be an independent factor for poor prognosis. Findings from this study provide a rationale for evaluating DNA damage response protein expression as a predictive marker for breast cancer treatment in future clinical trials with PARP inhibitors and immunotherapy.

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Compliance with ethical standards

Conflict of interest Authors have no conflict of interest to declare.

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