Breast Cancer Res Treat (2014) 148:211–220
DOI 10.1007/s10549-014-3127-z

EPIDEMIOLOGY

Dual specificity phosphatase 4 gene expression in association with triple-negative breast cancer outcome

Michelle L. Baglia · Qiuyin Cai · Ying Zheng · Jie Wu · Yinghao Su · Fei Ye · Ping-Ping Bao · Hui Cai · Zhiguo Zhao · Justin Balko · Wei Zheng · Wei Lu · Xiao-Ou Shu

Received: 14 July 2014 / Accepted: 3 September 2014 / Published online: 4 October 2014
© Springer Science+Business Media New York 2014

Abstract Triple-negative breast cancer (TNBC) is an aggressive cancer with limited treatment options. Dual specificity phosphatase 4 (*DUSP4*) has recently been suggested as a potential marker of chemotherapy resistance for TNBC. *DUSP4* gene expression levels were measured in breast cancer tissue from 469 TNBC patients aged 20–75 years who participated in the Shanghai Breast Cancer Survival Study, and their association with recurrence/breast cancer mortality and total mortality was evaluated. Information on breast cancer diagnosis, treatment, and disease progression was collected via medical chart review and multiple in-person follow-up surveys. A Cox regression model was applied in the data analyses. Over a median follow-up of 5.3 years (range: 0.7–8.9 years), 100 deaths and 92 recurrences/breast cancer deaths were documented. Expression levels of transcript variant 1 (NM_001394) and transcript variant 2 (NM_057158) of the *DUSP4* gene were studied and were highly correlated ($r = 0.76$). Low *DUSP4* expression levels, particularly of variant 1, were associated with both increased recurrence/breast cancer mortality and increased overall mortality. Hazard ratios with adjustment for age at diagnosis and TNM stage associated with below versus above the median expression level were 1.97 (95 % confidence interval (CI): 1.27–3.05) for recurrence/breast cancer mortality and 2.09 (95 % CI: 1.38–3.17) for overall mortality. Additional adjustment for expression levels of *MKI67* and *TP53*, common treatment types, breast cancer subtype, and grade did not materially alter the observed associations. Low *DUSP4* expression levels predict recurrence and mortality in TNBC patients independently from known clinical and molecular predictors.

Keywords DUSP4 · Triple-negative breast cancer · Recurrence · Mortality · Cohort study

Abbreviations

DUSP4	Dual specificity phosphatase 4
TNBC	Triple-negative breast cancer
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2

Introduction

Approximately 15–20 % of breast cancers in the United States are classified as triple-negative breast cancer (TNBC), characterized by minimal or no expression of estrogen receptors (ER) and progesterone receptors (PR) and the absence of overexpression of human epidermal growth factor 2 (HER2) [1]. TNBC is typically an aggressive cancer type displaying high rates of

M. L. Baglia · Q. Cai · J. Wu · Y. Su · F. Ye · H. Cai · Z. Zhao · W. Zheng · X.-O. Shu (✉)
Division of Epidemiology, Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, TN 37203, USA
e-mail: xiao-ou.shu@vanderbilt.edu

Y. Zheng · P.-P. Bao · W. Lu
Shanghai Municipal Center for Disease Control and Prevention, Shanghai 200336, People's Republic of China

J. Balko
Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

proliferation and metastasis and having generally poorer prognosis, particularly among those with advanced disease, as evidenced by increased recurrence and mortality compared with other breast cancer types [2]. Due to the lack of known therapeutic targets, such as ER, PR, and HER2 receptors, there are no specific therapies for TNBC tumors [3]. Thus, treatment options are limited. Chemotherapy is commonly used to treat TNBC, but a pathological complete response only occurs in approximately 30 % of cases; therefore, residual disease following treatment is common and accounts for the increased risk of metastatic recurrence and poorer outcomes [4].

Dual specificity phosphatase 4 (DUSP4) negatively regulates members of the mitogen-activated protein kinase (MAPK) superfamily, specifically extracellular-regulated kinase (ERK) activity, which is involved in cellular proliferation and survival [4–6]. Previous studies have suggested that DUSP4 inactivation and subsequent activation of the ERK pathway may play a role in tumor cell proliferation and growth [7, 8]. A recent study showed that low levels of *DUSP4* gene expression was associated with basal-like breast cancer (BLBC), high tumor cell proliferation after chemotherapy, dampened response to chemotherapy, and shorter recurrence-free survival [4]. Conversely, overexpression was correlated with increased chemotherapy-induced apoptosis. The authors suggested that the response to MEK inhibitors may be indicated by low *DUSP4* expression [9]. This finding was based on 111 total TNBC cases after neoadjuvant chemotherapy recruited from a single institute.

In the present study, we evaluated the association between *DUSP4* expression and overall and disease-free survival (DFS) in a cohort of 469 TNBC patients recruited into a population-based cohort study, the Shanghai Breast Cancer Survival Study (SBCSS).

Materials and methods

Study population

The SBCSS, described elsewhere previously, is a longitudinal, population-based cohort study of breast cancer survival in Shanghai, China [10]. Between March 2002 and April 2006, 5,042 women with incident breast cancer were identified from the population-based Shanghai Cancer Registry (response rate: 80 %). All participants were between 20 and 75 years of age at cancer diagnosis and were permanent residents of Shanghai. The study protocol was approved by the institutional review boards of Vanderbilt University and the Shanghai Municipal Center for Disease Control and Prevention, and all participants provided written informed consent.

Data collection

Participants were recruited to the study approximately 6 months following breast cancer diagnosis [mean (standard deviation):6.6 (0.7) months] [10]. In-person interviews, with trained interviewers, were conducted and information obtained included demographic characteristics, selected lifestyle factors, and clinical and treatment factors. Medical charts from the initial diagnostic hospital were reviewed to obtain clinical information, i.e., tumor characteristics, first-line treatments, and ER/PR status. HER2 status was measured in the Vanderbilt Molecular Epidemiology Lab using rabbit polyclonal antibody recognizing the HER2 cytoplasmic domain (DAKO, Cat# A0485, 1:100) following the DAKO Envision^{TM} kit protocol (DAKO, Cat# K4011) (details published elsewhere) [11]. Ten tumor sections (nine tumor sections (slides) in 5 micron and one section (slide) in 15 micron) were collected from the diagnostic hospitals for 4,036 SBCSS participants (80 %). Among these subjects, 525 were diagnosed with TNBC and included in the current study. Participants were followed up at 18, 36, and 60 months after breast cancer diagnosis to obtain information on disease progression and survival status. Survival information for participants lost to follow up was obtained using annual record linkage with the Shanghai Vital Statistics Registry.

Gene expression levels were measured in total RNA isolated from formalin-fixed paraffin-embedded (FFPE) breast cancer tissues using Nanostring technology. FFPE tissue sections and the H&E slides were reviewed by a study pathologist. Tumor tissue was selected and dissected to ensure that tissues contained >80 % tumor cells for total RNA extraction. Total RNA were isolated and purified using Qiagen's miRNeasy FFPE Kit (Qiagen, Valencia, CA), a kit specifically for purification of total RNA including miRNA from FFPE tissue sections, following the manufacturer's instructions. The quantity and quality of the RNA samples were checked by Nanodrop (E260, E260/E280 ratio, spectrum 220–320 nm) and by separation on an Agilent BioAnalyzer. We excluded 29 samples from the gene expression assay either due to the fact that tumor tissue was too small for RNA extraction (n = 19) or low RNA concentrations (n = 10), leaving 496 samples for expression assay.

Expression levels of transcript variant 1 (NM_001394) and transcript variant 2 (NM_057158) of the *DUSP4* gene were measured as a part of large gene expression effort. A custom-designed nCounter Gene Expression CodeSet profiling of 311 selected gene targets using the NanoString nCounter technology was performed following the NanoString standard protocol [12]. The selected gene panel included PAM50 genes, drug metabolism genes, reference genes, and a set of targeted genes based on

review and analysis of published data. The assay CV for the 2 *DUSP4* isoforms was 7.84 and 10.43 % based on eight QC samples (duplicated measurements of seven individual QC samples and ten repeated measurements of a pooled QC sample). The R package *NanoStringNorm (version 1.1.16)*, developed for normalization, diagnostics, and visualization of NanoString nCounter data, was used for quality assurance and data normalization. Specifically, quality control and raw data normalization were completed as follows: (1) adjust each sample's counts based on its relative value to the geometric mean of all samples to reduce technical variation; (2) correct background count level by subtracting each sample's count value from the mean +2 standard deviations of counts of the negatives controls; and (3) normalize for sample RNA content, i.e., "pipetting" fluctuations using the geometric mean of the expression levels of 5 pre-specified housekeeping genes (*ACTB*, *RPLP0*, *MRPL19*, *SF3A1*, and *PSMC4*). From 496 samples assayed, samples were excluded from statistical analysis if they met at least one of the following conditions: (1) positive normalization factor was outside of the range (0.3, 3) (N = 6); (2) samples with a background level > 3 standard deviations from the mean (N = 4); (3) samples with >90 % missing after background level correction (N = 11); and (4) samples with RNA content >3 standard deviations from the mean (N = 6), leaving a total of 469 samples with valid data for analysis. We applied the calling algorithm developed by Parker et al. to classify tumors into subgroups most resembling Basal-like, Luminal A, Luminal B, HER2-enriched, or Normal-like breast cancer based on PAM50 genes [13]. Briefly, for each sample, we calculated the Spearman's rank correlations between the gene set and the centroids of the five intrinsic subtypes: Luminal A, Luminal B, Basal-like, HER2-enriched, or Normal-like. These correlations were used as the distance metric, and all samples were then assigned to the subtype that had the minimum distance to the sample. To maintain the subtype structure in our study cohort, we did not apply the gene-wise centering during this calling process.

For the 469 samples included in the study, 418 were collected prior to chemotherapy [or the patient did not receive chemotherapy (N = 28)], 34 were collected after chemotherapy, and for the remaining 17 samples, the time line relevant to chemotherapy or radiotherapy could not be determined due to the overlapping of month of surgery and initiation of chemo- or radiotherapy (information on specific day of initiation was not collected).

Statistical analysis

The major endpoints for the study were any death for overall survival (OS) and cancer recurrence/metastasis or death related to breast cancer for DFS. The date of last in-person contact or June 2011 (6-months prior to date of latest record linkage), whichever was more recent, was used as the censor date for event-free subjects.

Clinical, treatment, demographic, and lifestyle factor variables were evaluated for their association with *DUSP4* expression levels (split at median expression level) using generalized linear models for continuous variables and χ^2 tests for categorical variables. Information on TNM stage was missing for 16 participants.

Using Cox proportional hazards models, the associations between *DUSP4* expression levels, analyzed both as a continuous variable and using median cutpoints, and recurrence and mortality were evaluated. The multivariate analyses were adjusted for age at diagnosis and TNM stage (five levels: stage 0–I (reference), IIA, IIB, III–IV, and missing). The associations were also examined by tumor molecular subtype.

Additional analyses stratified by chemotherapy (yes/no), type of chemotherapeutic drug (for the four most commonly used drugs), radiotherapy (yes/no), tamoxifen use, TNM stage, tumor grade, or use of vitamins C or E were performed to evaluate whether the relationship between *DUSP4* expression and recurrence and overall mortality was modified by these factors.

Statistical analyses were performed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC). The REMARK guidelines were followed in reporting the results of this study [14].

Results

The expression levels for the two isoforms for *DUSP4* were highly correlated ($r = 0.76$, $P < 0.0001$); the *DUSP4* variant 1 (NM_001394) was more predictive of outcome in this study and was chosen for more in-depth analyses as presented below. Over a median follow-up of 5.3 years (range: 0.7–8.9 years), 100 deaths and 92 recurrences/breast cancer mortalities were documented in this cohort of TNBC patients from the SBCSS cohort. DFS rate and OS were inversely associated with advanced stage disease and receiving radiotherapy (Table 1). Additionally, OS was inversely associated with menopausal status.

Participants with *DUSP4* (variant 1) expression levels below the median were significantly younger at diagnosis and were more likely to have a family history of breast cancer. No association was observed between *DUSP4* expression and other demographic and lifestyle factors. Breast cancer cases with *DUSP4* expression levels below the median were significantly more likely to have a higher grade tumor as compared to those with *DUSP4* expression levels above the median ($P < 0.0001$) (Table 2). Tumors

Table 1 Demographic and Clinical Predictors for Breast Cancer Survival for TNBC Patients in the Shanghai Breast Cancer Survival Study

Characteristics	N	5-Year disease-free survival			5-Year overall survival		
		Recurrences, No.	Rate, %[a]	*P*	Deaths, No.	Rate, %[a]	*P*
Age at diagnosis, y							
<40	34	9	71.4	0.53	10	70.5	0.08
40–49	168	25	83.3		22	86.7	
50–59	126	24	80.1		21	83.2	
≥60	141	31	76.2		31	78.0	
Education							
Elementary school or less	71	19	70.6	0.14	18	74.4	0.05
Middle school	156	33	77.4		26	83.3	
High or vocational school	168	22	86.0		24	85.5	
College or university	74	15	76.9		16	78.1	
Income							
<500	62	16	71.7	0.12	13	79.0	0.60
500–<700	70	18	72.3		15	78.5	
700–<1000	142	26	80.4		26	81.5	
1,000–<2000	146	23	82.7		22	84.8	
≥2000	48	6	85.9		8	83.1	
Body mass index							
<25	300	55	79.8	0.43	49	83.5	0.39
25–29.99	139	25	80.7		27	80.4	
≥30	30	9	68.5		8	72.8	
Menopausal status							
Premenopausal	213	34	82.5	0.27	31	85.3	0.04
Postmenopausal	256	55	76.8		53	79.2	
Tamoxifen use							
Yes	102	18	81.5	0.63	18	82.4	0.89
No	367	71	78.7		66	81.8	
TNM stage							
0–I	148	15	88.8	<.0001	15	89.7	<.0001
IIA	165	24	84.2		22	86.5	
IIB	93	25	70.0		26	71.8	
III–IV	47	22	50.8		19	59.6	
Unknown	16	3	80.4		2	87.5	
Grade							
1	56	6	88.1	0.31	3	94.6	0.02
2	151	29	78.9		27	82.1	
3	260	53	77.9		53	79.3	
Chemotherapy							
Yes	441	81	80.0	0.19	75	82.8	0.19
No	28	8	68.1		9	67.9	
Radiotherapy							
Yes	129	41	65.9	<.0001	39	69.5	<.0001
No	340	48	84.5		45	86.6	
Mastectomy							
Yes	447	84	79.6	0.41	80	81.9	0.68
No	22	5	74.4		4	81.8	
No. of live births							

Table 1 continued

Characteristics	N	5-Year disease-free survival			5-Year overall survival		
		Recurrences, No.	Rate, %[a]	*P*	Deaths, No.	Rate, %[a]	*P*
0	3	1	66.7	0.49	1	66.7	0.06
1	298	50	81.5		45	84.7	
2	91	21	75.7		20	78.0	
≥3	60	15	72.5		15	75.0	
Family history of BC							
Yes	34	8	73.4	0.28	6	82.2	0.75
No	435	81	79.8		78	81.9	

[a] Survival rate calculated using life table analysis method

Table 2 Clinical and Treatment Factors by *DUSP4* (variant 1) Expression

	<Median N (%)[a]	≥Median N (%)[a]	*P*
DUSP4 (variant 1) Expression			
TNM Stage			0.13
0-I	65 (43.9)	83 (56.1)	
IIA	85 (51.5)	80 (48.5)	
IIB	53 (57.0)	40 (43.0)	
III-IV	28 (59.6)	19 (40.4)	
Missing	4 (25.0)	12 (75.0)	
Grade			<.0001
1	11 (19.6)	45 (80.4)	
2	48 (31.4)	105 (68.6)	
3	176 (67.7)	84 (32.3)	
Chemotherapy	225 (51.0)	216 (49.0)	0.12
Radiotherapy	69 (53.5)	60 (46.5)	0.37
Surgery type			0.32
Mastectomy	227 (50.8)	220 (49.2)	
Conservation	3 (37.5)	5 (62.5)	
Unknown	4 (30.8)	9 (69.2)	
No surgery	1 (100.0)	0 (0.0)	
Tamoxifen use (baseline)	44 (43.1)	58 (56.9)	0.11
Subtype classification by PAM50			<.0001
Basal-like	160 (81.6)	36 (18.4)	
Her-2 Enriched	21 (33.3)	42 (66.7)	
Luminal A	17 (13.3)	111 (86.7)	
Luminal B	17 (40.5)	25 (59.5)	
Normal	20 (50.0)	20 (50.0)	

[a] Percentages shown are row percentages

with low *DUSP4* expression level were more likely to possess markers of the BLBC subtype ($P < 0.0001$).

Overall, the unadjusted 5-year disease-free and OS rates were 79.3 and 81.9 %, respectively, for this group of TNBC cases. *DUSP4* expression was inversely associated with the risk of recurrence/breast cancer mortality and total mortality (HR associated with per unit decrease of $\log_2$ transformed *DUSP4* expression level = 1.16, 95 % confidence interval (CI): 1.08, 1.25 and HR = 1.19, 95 % CI: 1.10, 1.28, respectively) (Table 3). Categorization of *DUSP4* expression into quartiles suggested that the median split could serve as an indicator of threshold effect. Compared to those with *DUSP4* expression above the median, those with *DUSP4* expression levels below the median had a 1.97-fold (95 % CI: 1.27, 3.05) increased risk of recurrence/breast cancer mortality and a 2.09-fold (95 % CI: 1.38, 3.17) increased risk of mortality. When the lower median was further categorized into two categories and compared to the upper median, the association was the strongest between those with the lowest *DUSP4* expression levels and the upper median for recurrence/breast cancer mortality (HR = 2.28, 95 % CI: 1.40, 3.72) and overall mortality (HR = 2.40, 95 % CI: 1.50, 3.84); the HRs for the upper half of the lower median were 1.66 (95 % CI: 0.97, 2.81) for recurrence/breast cancer mortality and 1.79 (95 % CI: 1.08, 2.95) for overall mortality as compared to the upper median. Those with the lowest *DUSP4* expression levels also had the lowest disease-free and OS rates (69.6 and 72.6 %, respectively). Participants with stage 0 tumors (n = 7) had *DUSP4* expression levels greater than the median (mean = 9.72), and the participant with a stage IV tumor (n = 1) had a *DUSP4* expression level below the median (*DUSP4* expression = 6.69). Excluding participants with stage 0 or stage IV tumors did not materially change the observed results (HR for the upper median compared to the lower median was 1.88, 95 % CI: 1.22, 2.90 for recurrence/breast cancer mortality and 1.99, 95 % CI: 1.31, 3.01 for overall mortality; HR for the continuous DUSP4 measure was 1.16, 95 % CI: 1.08, 1.25 for recurrence/breast cancer mortality and 1.19, 95 % CI: 1.10, 1.28 for overall mortality). Figure 1 shows the Kaplan–Meier DFS curve by the lower and upper median of *DUSP4* expression.

DUSP4 expression was inversely correlated with *MKI67* gene expression ($r = -0.24$, $P < 0.0001$), an indicator for

Table 3 Disease-Free and Overall Survival Analysis by *DUSP4* (variant 1) Expression

	Disease-free survival			Overall survival		
	5-yr DFS rate, %[a]	HR (95 % CI)[b]	*P*	5-yr OS rate, %[a]	HR (95 % CI)[b]	*P*
Model 1[c]						
DUSP4	79.3	1.16 (1.08, 1.25)	0.0002	81.9	1.19 (1.10, 1.28)	0.00001
Age at diagnosis		1.01 (0.99–1.03)	0.29		1.02 (1.00 - 1.04)	0.03
Stage IIA		1.48 (0.78–2.79)	0.23		1.47 (0.80 - 2.68)	0.21
Stage IIB		2.86 (1.51–5.44)	0.001		2.95 (1.62 - 5.35)	0.0004
Stage III and IV		5.93 (3.09–11.39)	<.00001		5.74 (3.06 - 10.80)	<.00001
Stage missing		2.26 (0.65–7.87)	0.20		1.46 (0.33 - 6.36)	0.62
Model 2[d]						
DUSP4 (≥ median)	85.4	Reference		88.3	Reference	
DUSP4 (< median)	73.3	1.97 (1.27, 3.05)	0.002	75.6	2.09 (1.38, 3.17)	0.0005
Age at diagnosis		1.01 (0.99–1.03)	0.22		1.02 (1.00–1.04)	0.02
Stage IIA		1.46 (0.77–2.75)	0.25		1.44 (0.79–2.64)	0.23
Stage IIB		2.76 (1.45–5.27)	0.002		2.82 (1.55–5.14)	0.0007
Stage III and IV		5.74 (2.99–11.03)	<.00001		5.24 (2.80–9.82)	<.00001
Stage missing		2.19 (0.63–7.62)	0.22		1.40 (0.32–6.10)	0.66
Model 3[e]						
DUSP4 (≥ median)	85.4	Reference		88.3	Reference	
Upper half of lower median	76.9	1.66 (0.97, 2.81)	0.06	78.6	1.79 (1.08, 2.95)	0.02
Lower half of lower median	69.6	2.28 (1.40, 3.72)	0.0009	72.6	2.40 (1.50, 3.84)	0.0003
Age at diagnosis		1.01 (0.99–1.03)	0.22		1.02 (1.00–1.04)	0.02
Stage IIA		1.44 (0.76–2.73)	0.26		1.43 (0.78–2.61)	0.25
Stage IIB		2.75 (1.45–5.25)	0.002		2.79 (1.53–5.08)	0.0008
Stage III and IV		5.64 (2.93–10.84)	<.00001		5.21 (2.78–9.76)	<.00001
Stage missing		2.19 (0.63–7.63)	0.22		1.38 (0.32–6.04)	0.67

[a] Unadjusted, mean (se)

[b] Adjusted for age at diagnosis and TNM stage

[c] DUSP4 treated as continuous variable

[d] DUSP4 analyzed using median cutpoint, upper median as reference

[e] DUSP4 analyzed using median cutpoint, lower median split, upper median as reference

higher tumor cell proliferation [15]. However, controlling for *MKI67* gene expression level did not alter the association between *DUSP4* and recurrence and mortality (lower median compared to upper: HR = 2.00, 95 % CI: 1.28, 3.15 and HR = 2.13, 95 % CI: 1.38, 3.28, respectively). Furthermore, *DUSP4* expression was positively associated with expression levels of *ESR1*, *PGR*, and *ERBB2*. Controlling for *ESR1* expression changed the association with *DUSP4* expression for recurrence/breast cancer mortality to HR = 2.21 (95 % CI: 1.37, 3.55). The *DUSP4* association was not materially changed when controlling for expression levels of *PRG and ERRB2*. *DUSP4* expression was inversely associated with expression level of the *MYC* ($r = -0.19$, $P < 0.0001$) and positively associated with expression level of the *MAPT* ($r = 0.37$, $P < 0.0001$), genes which are both in the ERK pathway; controlling for these genes did not alter the results materially (data not shown). No correlation between *DUSP4* and *TP53* expression was observed, and controlling for *TP53* did not alter the observed results.

Despite that tumors with markers of the basal-like subtype were more likely to have low *DUSP4* expression, analysis stratified by molecular subtype based on PAM50 genes showed that *DUSP4* expression was associated with overall mortality and recurrence/breast cancer mortality in basal-like (HR for continuous *DUSP4* measure = 1.22, 95 % CI: 1.05, 1.41 for overall mortality and HR = 1.16, 95 % CI: 1.01, 1.33 for recurrence/breast cancer mortality) and non-basal-like breast cancer subtypes (HR = 1.16, 95 % CI: 1.05, 1.30 for overall mortality and HR = 1.14, 95 % CI: 1.02, 1.28 for recurrence/breast cancer mortality).

No interactions with TNM stage (0-IIA vs IIB-IV), tumor grade (1 and 2 vs 3), chemotherapy, radiotherapy, tamoxifen use, and use of vitamin C or E (ever vs never)

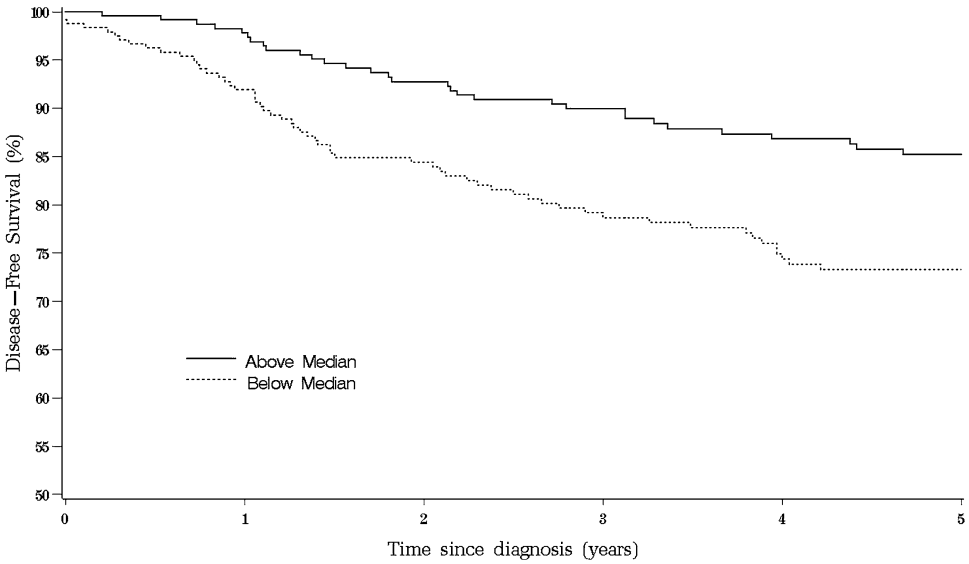

Fig. 1 Survival time since diagnosis in years is on the *x*-axis and disease-free survival as a percentage is on the *y*-axis. Kaplan–Meier curves, comparing those with *DUSP4* (variant 1) expression levels *above* (n = 234) and *below* (n = 235) the median, show a significantly worse prognosis for patients with *DUSP4* expression levels below the median ($P = 0.002$)

were observed for *DUSP4* expression. We evaluated the most commonly used chemotherapy drugs (5-fluorouracil, cyclophosphamide, methotrexate, and epirubicin) as potential effect measure modifiers (Table 4); *DUSP4* expression, analyzed continuously, was more predictive of DFS in patients who received methotrexate (HR for continuous *DUSP4* measure = 1.32, 95 % CI: 1.11, 1.58) than those who did not (HR = 1.10, 95 % CI: 1.01, 1.21) ($P = 0.02$ for interaction). We did not find that the *DUSP4* associations varied by other common chemotherapy drugs or radiotherapy.

Discussion

The results from this large, population-based cohort study show that low *DUSP4* gene expression is associated with increased mortality and breast cancer recurrence among patients with TNBC. Although low *DUSP4* expression was more common in tumors with BLBC markers and was inversely associated with *MKI67* and *MYC* gene expressions, its association with breast cancer outcomes was independent of these factors.

A member of the dual specificity protein phosphatase family, *DUSP4* dephosphorylates the phosphoserine/threonine and phosphotyrosine residues of its target kinase, resulting in an inactivation of the kinase [16]. DUSP4 regulates cell proliferation and survival by negatively regulating members of the MAPK superfamily through inactivation of ERK1, ERK2, and JNK [6]. DUSP4 downregulation results in Ras–ERK pathway activation. The Ras signaling pathways play a substantial role in controlling normal cell growth and, when activated, lead to uncontrolled cell growth [17]. Activation of this pathway may in turn lead to reduced response to chemotherapy [4, 9]. If this hypothesis is correct, *DUSP4* gene expression quantification may be useful in predicting activity of the Ras–ERK pathway [9]. MEK inhibitors could be used in combination with chemotherapy agents to potentially improve response to chemotherapy [9].

In a recent study using Nanostring technology to profile 49 breast cancer tissue samples, primarily TNBC, surgically resected after neoadjuvant chemotherapy, Balko et al. found that low *DUSP4* expression correlated with high tumor cell proliferation following neoadjuvant chemotherapy and BLBC tumors [4]. Pathological response to chemotherapy was inversely associated with *DUSP4* expression, and the authors concluded that the Ras–ERK pathway may be activated by downregulation of *DUSP4* in BLBC, resulting in an attenuated response to chemotherapy. The authors further demonstrated that treatment with a MEK inhibitor enhanced sensitivity to the chemotherapeutic drug, docetaxel, in vivo. The authors concluded that *DUSP4* may be a potential marker of resistance to chemotherapy drugs.

Similarly, in our study, low *DUSP4* expression was more common in BLBC tumors and was associated with increased recurrence/breast cancer mortality and increased total mortality. Tumors with BLBC subtype markers had increased recurrence/breast cancer mortality and total mortality compared with tumors without these markers. However, adjustment for *DUSP4* expression attenuated, but did not completely eliminate, the survival disadvantage for BLBC tumors, suggesting that *DUSP4* down regulation may only be one of the mechanisms responsible for the aggressive clinical behavior of BLBC. In our study, *DUSP4* expression was inversely related to *MKI67* and *MYC* expression and positively associated with *ERS1, PR, ERBB2,* and *MAPT* expression, but not TP53 expression.

Table 4 *DUSP4* (variant 1) Expression–Treatment Interactions in Disease-Free and Overall Survival

Treatment	Disease-free survival				Overall survival			
	Events/ total	HR[a,b] (95 % CI)	HR[a,c] (95 % CI)	*P* for interaction[b]	Events/ Total	HR[a,b] (95 % CI)	HR[a,c] (95 % CI)	*P* for interaction[b]
Chemotherapy				0.88				0.82
Yes	84/441	1.16 (1.07, 1.26)	1.89 (1.20, 2.99)		91/441	1.18 (1.09, 1.28)	1.96 (1.27, 3.03)	
No	8/28	1.22 (0.88, 1.69)	2.34 (0.46, 11.94)		9/28	1.28 (0.94, 1.76)	2.74 (0.57, 13.31)	
Radiotherapy				0.32				0.42
Yes	41/129	1.10 (0.96, 1.27)	1.57 (0.83, 2.98)		42/129	1.12 (0.96, 1.30)	1.39 (0.74, 2.59)	
No	51/340	1.19 (1.08, 1.31)	2.22 (1.22, 4.04)		58/340	1.21 (1.11, 1.33)	2.65 (1.51, 4.66)	
5-fluorouracil				0.52				0.60
Yes	61/354	1.17 (1.07–1.29)	1.96 (1.14–3.37)		67/354	1.20 (1.09–1.31)	1.94 (1.16–3.25)	
No	31/115	1.14 (1.00–1.29)	2.09 (0.98–4.44)		33/115	1.16 (1.02–1.32)	2.47 (1.20–5.08)	
Cyclophosphamide				0.93				0.73
Yes	62/337	1.15 (1.04–1.27)	1.89 (1.11–3.22)		67/337	1.19 (1.08–1.30)	2.03 (1.22–3.41)	
No	30/132	1.17 (1.03–1.33)	2.07 (0.95–4.47)		33/132	1.17 (1.03–1.33)	2.21 (1.08–4.51)	
Methotrexate				0.02				0.06
Yes	19/100	1.32 (1.11–1.58)	5.53 (1.58–19.35)		23/100	1.31 (1.12–1.53)	6.01 (2.01–17.94)	
No	73/369	1.10 (1.01–1.21)	1.56 (0.97–2.51)		77/369	1.13 (1.04–1.24)	1.56 (0.98–2.48)	
Epirubicin				0.62				0.82
Yes	56/254	1.13 (1.03–1.25)	1.98 (1.13–3.49)		56/254	1.19 (1.07–1.31)	1.83 (1.05–3.20)	
No	36/215	1.19 (1.03–1.37)	1.82 (0.91–3.65)		44/215	1.18 (1.04–1.34)	2.44 (1.29–4.59)	

[a] Adjusted for age at diagnosis and TNM stage

[b] DUSP4 expression analyzed continuously

[c] DUSP4 expression analyzed at median cutpoint

Additionally, adjustment for *TP53*, *MKI67*, *ESR1*, *PGR*, and *ERBB2* gene expression level did not change the association. These results suggest that the association of *DUSP4* with recurrence/survival is independent of the molecular subtypes of breast cancer as defined by PAM50 and the known molecular prognostic markers for TNBC.

While our study only involved TNBC, deregulation of *DUSP4* on cancer prognosis may not be confined to TNBC. Aberrations in many of the above-mentioned DUSP4-correlated genes are commonly seen in other types of breast cancer [18–20]. In our study, *DUSP4* expression was associated with breast cancer outcomes for non-basal-like TNBC, including those that expressed luminal A or B signature genes. Using the publicly available Dutch Cancer Institute (NKI) breast cancer dataset (https://www.synapse.org/#!Synapse:syn4517), we found that DUSP4 expression was associated with outcomes of ER + tumors (HR associated with per unit decrease of $\log_2$ transformed *DUSP4* expression level = 1.45, 95 % CI: 1.12, 1.86 for recurrence and HR = 1.57, 95 % CI: 1.13, 2.18 for overall mortality, after adjustment for age at diagnosis and tumor grade) [21]. These results call for more investigation on the role of DUSP4 in other major breast cancer subtypes.

It is important to note the differences in populations used in our study compared with the previous study by Balko et al. The previous findings were observed in breast cancer tissue taken from European-ancestry women, whereas our finding of a similar association in an Asian-ancestry population suggests that the *DUSP4* association is independent of race and ethnicity. Furthermore, all patients in the study by Balko et al. were treated with the chemotherapy drug, docetaxel, before tumor resection. The percentage of patients who received docetaxel in our population was low (3.4 %), and the majority of patients received other chemotherapy agents or combinations of agents, including 5-fluorouracil (75.5 %), cyclophosphamide (71.9 %), epirubicin (54.2 %), and methotrexate (21.3 %). Additionally, the majority of participants in our study had not received chemotherapy prior to surgery when the tissue sample for gene expression quantification was resected.

Our data show that *DUSP4* expression was predictive of outcomes among many different groups, including those whose *DUSP4* expression level was quantified prior to chemotherapy treatment and those who did not receive chemotherapy treatment at all (although the sample size for the latter group was small). There were some indications that *DUSP4* expression was more predictive of outcome among those who received certain types of chemotherapy; however, the interaction was only significant for those who took methotrexate. Taken together, our study suggests that *DUSP4* expression may predict breast cancer outcomes beyond its association with response to chemotherapy as previously suggested.

The present study has several strengths. The data come from a large, population-based cohort study with comprehensive information collected on potential covariates. Information on clinical and disease factors was verified through medical chart review, which increased the validity of the data. A limitation of our study is that the timing of biological sampling with respect to chemotherapy treatment could not be determined for 17 patients. However, the significant association between DUSP4 gene expression and recurrence/breast cancer mortality and overall mortality was similar when only those with tumor tissue samples taken prior to chemotherapy initiation were included in the analyses.

Further research is needed to better understand the biological mechanism(s) underlying the association of *DUSP4* expression with TNBC outcomes and to investigate the role of *DUSP4* in the prognosis of various molecular subtypes of breast cancer. The gene–drug interaction, in particular the role of *DUSP4* expression on chemotherapy resistance, may be better evaluated in a randomized clinical trial.

In summary, our study confirmed that low *DUSP4* expression was associated with increased recurrence/breast cancer mortality and increased total mortality among TNBC patients. This association was independent of markers of BLBC and other known clinical and molecular predictors.

Acknowledgments We thank Regina Courtney and Bo Huang for their help with RNA sample preparation. Sample preparation was conducted at the Survey and Biospecimen Shared Resources, which is supported in part by the Vanderbilt-Ingram Cancer Center (P30CA068485). NanoString nCounter assays were conducted at the University of Miami Sylvester Comprehensive Cancer Center. This study was supported by grants from the US Department of Defense (DOD) Breast Cancer Research Program (DAMD 17-02-1-0607 to X.-O. Shu) and the National Institutes of Health (NIH; R01 CA118229 to X.-O. Shu, P50CA098131 to C. Arteaga). Michelle Baglia is funded by a Clinical and Translational Science Award (CTSA) TL1 fellowship.

Ethical Standards This study complies with the current laws of the country in which they were performed.

Conflict of interest The authors have no conflicts of interest to disclose.

References

1. Pal SK, Childs BH, Pegram M (2011) Triple negative breast cancer: unmet medical needs. Breast Cancer Res Treat 125(3):627–636. doi:10.1007/s10549-010-1293-1
2. Ossovskaya V, Wang Y, Budoff A, Xu Q, Lituev A, Potapova O, Vansant G, Monforte J, Daraselia N (2011) Exploring molecular pathways of triple-negative breast cancer. Genes cancer 2(9):870–879. doi:10.1177/1947601911432496
3. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA (2007) Triple-

negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 13(15 Pt 1):4429–4434. doi:10.1158/1078-0432.CCR-06-3045
4. Balko JM, Cook RS, Vaught DB, Kuba MG, Miller TW, Bhola NE, Sanders ME, Granja-Ingram NM, Smith JJ, Meszoely IM, Salter J, Dowsett M, Stemke-Hale K, Gonzalez-Angulo AM, Mills GB, Pinto JA, Gomez HL, Arteaga CL (2012) Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4 deficiency as a mechanism of drug resistance. Nat Med 18(7):1052–1059. doi:10.1038/nm.2795
5. Jeffrey KL, Camps M, Rommel C, Mackay CR (2007) Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discovery 6(5):391–403. doi:10.1038/nrd2289
6. Lawan A, Al-Harthi S, Cadalbert L, McCluskey AG, Shweash M, Grassia G, Grant A, Boyd M, Currie S, Plevin R (2011) Deletion of the dual specific phosphatase-4 (DUSP-4) gene reveals an essential non-redundant role for MAP kinase phosphatase-2 (MKP-2) in proliferation and cell survival. J Biol Chem 286(15):12933–12943. doi:10.1074/jbc.M110.181370
7. Waha A, Felsberg J, Hartmann W, von dem Knesebeck A, Mikeska T, Joos S, Wolter M, Koch A, Yan PS, Endl E, Wiestler OD, Reifenberger G, Pietsch T (2010) Epigenetic downregulation of mitogen-activated protein kinase phosphatase MKP-2 relieves its growth suppressive activity in glioma cells. Cancer Res 70(4):1689–1699. doi:10.1158/0008-5472.CAN-09-3218
8. Cagnol S, Rivard N (2013) Oncogenic KRAS and BRAF activation of the MEK/ERK signaling pathway promotes expression of dual-specificity phosphatase 4 (DUSP4/MKP2) resulting in nuclear ERK1/2 inhibition. Oncogene 32(5):564–576. doi:10.1038/onc.2012.88
9. Rottenberg S, Jonkers J (2012) MEK inhibition as a strategy for targeting residual breast cancer cells with low DUSP4 expression. Breast cancer res 14(6):324. doi:10.1186/bcr3327
10. Shu XO, Zheng Y, Cai H, Gu K, Chen Z, Zheng W, Lu W (2009) Soy food intake and breast cancer survival. JAMA 302(22):2437–2443. doi:10.1001/jama.2009.1783
11. Su Y, Zheng Y, Zheng W, Gu K, Chen Z, Li G, Cai Q, Lu W, Shu XO (2011) Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: a population-based cohort study. BMC Cancer 11:292. doi:10.1186/1471-2407-11-292
12. Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, Fell HP, Ferree S, George RD, Grogan T, James JJ, Maysuria M, Mitton JD, Oliveri P, Osborn JL, Peng T, Ratcliffe AL, Webster PJ, Davidson EH, Hood L, Dimitrov K (2008) Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 26(3):317–325. doi:10.1038/nbt1385
13. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27(8):1160–1167. doi:10.1200/JCO.2008.18.1370
14. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2006) REporting recommendations for tumor MARKer prognostic studies (REMARK). Breast Cancer Res Treat 100(2):229–235. doi:10.1007/s10549-006-9242-8
15. Guarneri V, Piacentini F, Ficarra G, Frassoldati A, D'Amico R, Giovannelli S, Maiorana A, Jovic G, Conte P (2009) A prognostic model based on nodal status and Ki-67 predicts the risk of recurrence and death in breast cancer patients with residual disease after preoperative chemotherapy. Ann Oncol Off J European Soc Med Oncol 20(7):1193–1198. doi:10.1093/annonc/mdn761
16. Peng DJ, Zhou JY, Wu GS (2010) Post-translational regulation of mitogen-activated protein kinase phosphatase-2 (MKP-2) by ERK. Cell Cycle 9(23):4650–4655
17. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 3(1):11–22. doi:10.1038/nrc969
18. Byler S, Goldgar S, Heerboth S, Leary M, Housman G, Moulton K, Sarkar S (2014) Genetic and epigenetic aspects of breast cancer progression and therapy. Anticancer Res 34(3):1071–1077
19. Sabatier R, Goncalves A, Bertucci F (2014) Personalized medicine: present and future of breast cancer management. Crit Rev Oncol Hematol 91(3):223–233. doi:10.1016/j.critrevonc.2014.03.002
20. Baquero MT, Lostritto K, Gustavson MD, Bassi KA, Appia F, Camp RL, Molinaro AM, Harris LN, Rimm DL (2011) Evaluation of prognostic and predictive value of microtubule associated protein tau in two independent cohorts. Breast Cancer Res 13(5):R85. doi:10.1186/bcr2937
21. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347(25):1999–2009. doi:10.1056/NEJMoa021967