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SHP2 expression is an independent negative prognostic factor in human breast cancer

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Abstract

Aims—Src-homology-phosphotyrosyl-phosphatase 2 (SHP2) is a ubiquitously expressed phosphatase that plays an essential role in the downstream signaling pathways of multiple growth factor receptors, thus representing a potential target for cancer therapy. Recent studies suggest that SHP2 contributes to tumor initiation, progression and metastasis in breast cancer, yet the impact of SHP2 expression on prognosis in human breast cancer has not been evaluated.

Methods and Results—To further explore the role of SHP2 in breast cancer, we conducted an immunohistochemical study on a tissue microarray encompassing 1401 formalin-fixed breast cancer specimens with detailed clinical annotation and outcomes data. 651 of 1401 (46%) evaluable breast cancers were positive for SHP2. SHP2 expression was positively associated with tumor grade, lymph node status, and tumor stage. In univariate survival analysis, cases with SHP2 expression had a significantly worse overall survival (OS). In multivariate analysis, SHP2 remained an independent negative prognostic factor for OS. SHP2 expression was a negative prognostic factor for OS in the luminal A and the luminal B HER2[−] intrinsic subtypes.

Conclusions—Our data demonstrate for the first time that SHP2 is an independent predictor of survival in breast cancer, suggesting that SHP2 may be a potential target for therapy.

Keywords

SHP2; PTPN11; breast cancer; prognostic factor

INTRODUCTION

Protein tyrosine phosphatases (PTP) play a major role in a broad range of cellular functions, including proliferation, differentiation, intercellular communication and motility ^{1,2}.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Multiple studies indicate that PTPs function in collaboration with protein tyrosine kinases in regulating the levels of cellular tyrosine phosphorylation, thereby modulating signaling pathways in diverse physiological processes ^{1, 2}. Src homology phosphotyrosyl phosphatase 2 (SHP2) is a ubiquitously expressed cytoplasmic enzyme that plays an important role in the downstream signaling pathways of multiple growth factor receptors such as the epidermal growth factor receptor (EGFR) and the platelet derived growth factor receptor (PDGFR) ³.

Although it is not completely understood how SHP2 modulates these signaling cascades, activation of growth factor receptors, including EGFR, results in SHP2 phosphorylation, and extracellular signal regulated protein kinase (ERK) activation ⁴. ERK in turn phosphorylates different substrates in the cytosol and traverses the nuclear membrane to activate transcription factors ⁵.

There is accumulating evidence suggesting that SHP2 may play a crucial role in cancer. SHP2 activation can occur either downstream of oncoproteins or by mutations ⁶. Germline mutations in the *PTPN11* gene which encodes SHP2 are reported in about half of the patients with Noonan Syndrome, an autosomal dominant disorder whose features include an increased risk of myeloproliferative disorders ⁷. Somatic gain-of-function mutations of the *PTPN11* gene have also been identified in patients with juvenile myeloid leukemia as well as in some cases of lung adenocarcinomas, colon cancers, thyroid cancers, melanomas and neuroblastomas ⁷⁻⁹. These genetic observations identified *PTPN11* as the first oncogene in the PTP superfamily ¹. Activation of the Ras-ERK and the PI3K-AKT signaling pathways are thought to be the principal transforming effect of mutant SHP2 ^{3, 10}. Since wildtype SHP2 modulates important signaling events downstream of various growth factor receptors and proto-oncogenes, *PTPN11* is now considered a bona fide oncogene ⁷ and thus represents a potential target for cancer therapy ¹⁰.

Of note, there is also evidence that SHP2 may act as a tumor suppressor in liver cancer ¹¹. Deletion of *PTPN11* in hepatocytes leads to development of hepatocellular tumors in aged mice, and SHP2 ablation increases development of diethylenetriamine-induced hepatocellular carcinoma in a mouse model ¹¹.

Several studies to date suggest a role for SHP2 in breast cancer. An immunohistochemistry study by Zhou et al. ³ reported overexpression of SHP2 in 72% of invasive ductal breast cancers and an association between SHP2 expression, tumor grade, and lymph node metastasis. The authors demonstrated that the immunohistochemical method they used was able to discriminate between SHP2 overexpression in breast cancer compared to normal breast tissue, suggesting that immunohistochemistry is a robust technique for evaluating SHP2 expression ³. SHP2 expression was also reported to be associated with both Human Epidermal Growth Factor Receptor 2 (HER2) expression, as well as with estrogen receptor (ER) expression and progesterone receptor (PR) expression ³. Consistent with these findings are two studies suggesting that SHP2 plays a role in HER2-induced breast cancer signaling/transformation ^{3, 12, 13}. In the first study, inhibition of SHP2 in HER2-positive breast cancer cell lines abrogated survival signaling, resulting in a conversion to a normal breast epithelial phenotype ¹³. This study also showed that SHP2 promotes epithelial-mesenchymal transition in breast cancer cell lines with downregulation of E-cadherin and upregulation of

fibronectin and vimentin¹³. In the second study, the authors showed that HER2 is a direct substrate for SHP2 and that dephosphorylation of a single autophosphorylation site in HER2 by SHP2 leads to enhanced HER2 signaling and cell transformation¹². To date no mutations or amplifications of *PTPN11* in breast cancer cell lines have been identified. However, the gene encoding the SHP2-activating protein growth factor receptor bound 2-associated protein 2 (GAB2) is amplified and overexpressed in 10–15% of human breast cancers^{14, 15}.

Recently, Aceto et al. confirmed an important role for SHP2 in tumor initiation, progression and metastasis in HER2⁺ and triple-negative breast cancers⁶. The authors demonstrated that specific ablation of SHP2 led to reduced breast cancer invasion *in vitro*, as well as decreased tumor growth and lung metastases in a mouse model. They also found a high expression of SHP2 protein by immunohistochemistry in 91% of breast cancers, but SHP2 expression did not correlate with histological subtype or clinicopathological parameters. Moreover, they identified a set of “SHP2 signature genes” that are activated in the presence of SHP2 signaling in a subset of human breast cancers and are associated with invasive behavior and poor prognosis⁶.

Despite this interest in defining the role of SHP2 in cancer biology at the molecular level, the impact of SHP2 expression on prognosis in human breast cancer has not been studied. To address this issue and to further explore the role of SHP2 in human breast cancer, we performed immunohistochemistry studies on a breast cancer tissue microarray (TMA) encompassing a total of 1401 formalin fixed breast cancer specimens with detailed clinical annotation and outcomes data.

MATERIALS AND METHODS

Tissue microarray

We used a tissue microarray (TMA) encompassing 2020 breast cancer tissue punches from 1579 formalin-fixed and paraffin-embedded tumor samples, which were collected from patients diagnosed with primary breast cancer between 1985 and 2007 at the Institute for Pathology, University of Basel and the Viollier Institute in Basel, Switzerland. The tissue samples were brought into a TMA format as previously described¹⁶. In brief, 0.6 mm tissue cylinders were punched out of donor tumor tissue blocks and transferred into a recipient paraffin block using a semiautomated tissue arrayer. Each TMA contained a number of tumor punches ranging from 159 to 522. Histopathological data was obtained from the pathology reports, and raw patients survival data was obtained from the Cancer Registry of Basel or from the patient's attending physicians. Retrieval of tissue and clinical data was performed according to the regulations of the local institutional review boards and data safety laws with specific regard to ethical standards and patient confidentiality.

Immunohistochemistry

SHP2 immunohistochemistry was performed using standard techniques. Briefly, 4 µm sections of the TMA blocks were incubated overnight with primary anti-SHP2 antibody (LifeSpan Biosciences, Inc., Seattle, WA, Catalog # LS-B542) after heat induced antigen

retrieval with Citrate buffer at pH 6. Standard DAB technique (Dako EnVision⁺ System; labelled polymer anti-rabbit followed by Liquid DAB⁺ substrate chromogen system) was employed for immunostaining. Counterstaining was performed with Harris hematoxylin solution.

Consistent with the known intracellular localization of SHP2, breast cancers with positive SHP2 staining showed a cytoplasmic distribution of SHP2. Using the same scoring system as described by Zhou et al.³, strong cytoplasmic reactivity of > 10% of all tumor cells was designated as 3+, moderate reactivity as 2+, and faint reactivity as 1+ (Figure 1). Faint reactivity not discernible above background or absence of any staining was considered negative for SHP2 expression. A reactivity of 2+ and 3+ was considered positive for SHP2 expression. Microscopically normal portions of breast specimens were used as controls.

The staining intensity of ER, PR, and HER2 was scored as described previously¹⁷. In brief, tumors were considered positive for ER if they showed nuclear staining in more than 10% of tumor cells, with an intensity score between 1 and 3.

Statistical analysis

The primary outcome was overall survival (OS), which was defined as the time from the first operation to death due to any cause. Survivors were censored at the date of last contact. The distributions of patient and clinical characteristics between SHP2-positive and negative tumors were compared using Chi-square test, Wilcoxon rank sum test, or two-sample t-test as appropriate. Survival curves by SHP2 status were estimated using the Kaplan-Meier product-limit method and compared by log-rank test. Univariate Cox proportional hazard models were fit to identify factors significantly related to overall survival. To assess whether SHP2 was an independent predictor of survival, a multivariate Cox model was constructed to adjust other patient/clinical characteristics that were significant in the univariate analyses. Two-way interaction terms between SHP2 and other factors in the multivariate Cox model were also assessed. All analyses were two-sided and significance was set at a *p* value of 0.05. Statistical analyses were performed using SAS (SAS Institutes, Cary, NC).

RESULTS

A total of 1401 cases were evaluable for this study. The mean follow up time was 80.8 months (range 1 to 263 months), and the mean age of the patients was 63 years (range 27 to 101 years). Demographic information for the patients included in this study can be found in Table 1.

Using the afore mentioned scoring system, a total of 651 of 1401 (46%) evaluable primary breast cancers were positive for SHP2.

SHP2 expression showed a positive correlation with AJCC primary tumor classification, lymph node status, and tumor grade (Table 2). SHP2 expression also varied significantly across the different histologic types of breast cancer, being highest in the invasive ductal cancers (47.3%) and lowest in cribriform cancers (31.7%, *p* = 0.0250) (Table 3).

Breast cancer intrinsic subtypes were originally defined by gene expression profiling¹⁸ but can be approximated using immunohistochemistry for ER, PR, Ki-67 and HER2¹². Breast cancer intrinsic subtypes are known to have distinct epidemiological risk factors, and vary in terms of prognosis, and response to therapy¹². There was no significant difference in SHP2 expression across the different breast cancer intrinsic subtypes ($p = 0.9108$, Table 4), as defined by the St. Gallen consensus conference¹⁹.

SHP2 showed a positive correlation with expression of the protein tyrosine phosphatase 1B (PTP1B) ($p = 0.026$) (data not shown). However, no significant association was found with ER or PR receptor status (data not shown), or HER2 status (Table 2).

Univariate survival analyses showed that breast cancers with positive SHP2 expression had a significantly worse overall survival (OS) (HR = 1.458, $p < 0.0001$). In the different breast cancer intrinsic subtypes, SHP2 expression proved to be a negative prognostic factor for OS in univariate analysis in the luminal A (HR = 1.813, $p = 0.0323$) and the luminal B HER2⁻ subtypes (HR = 1.572, $p = 0.0005$). No difference in OS with SHP2 expression was found in the Luminal B HER2⁺ subtype (HR = 1.346, $p = 0.237$), HER2 subtype (HR = 1.457, $p = 0.1765$), or basal-like subtype (HR = 1.076, $p = 0.7024$) (Figure 2 and Table 5).

In multivariate analysis, after adjusting for age, grade, tumor stage, lymph node status, and intrinsic subtype, SHP2 remained an independent negative prognostic factor for OS (HR = 1.239, $p = 0.0177$). The 2-way interaction term between SHP2 and intrinsic subtype was not significant ($p = 0.91$) (Table 6).

DISCUSSION

We examined the expression of SHP2 in a large cohort of 1401 primary breast cancers and demonstrate for the first time that expression of SHP2 is an independent negative prognostic factor for overall survival in human breast cancer. Our study has important implications as small molecule inhibitors targeting SHP2 are currently under development.

In our study, SHP2 expression in human breast cancer was lower than previously reported. We observed SHP2 expression in 651 of 1401 cases (46%), compared to 72% in the study by Zhou et al.³, and 91% in the study by Aceto et al.⁶. Of note, all three studies used the same scoring system, but different antibodies. The antibody we used is directed against the N-Terminus of SHP2 and has been validated for immunohistochemistry. Of note, we also stained normal breast tissue, which showed faint epithelial staining that was barely discernible beyond background (data not shown). This provides additional evidence that the antibody we used is suitable for the detection of SHP2 expression in breast cancer.

Consistent with Zhou et al., we found a positive association between SHP2 expression, tumor grade, and lymph node metastasis in breast cancer, although to a lesser extent³. Several studies have demonstrated that SHP2 promotes cell migration and invasion^{6, 22}. Consistent with these findings, SHP2 expression was highly associated with lymph node metastasis in our study ($p=0.0263$).

In contrast with Zhou et al.³, there was no association between SHP2 expression and ER or PR expression, and SHP2 expression was not significantly different across the different intrinsic subtypes, underscoring the fact that SHP2 represents a broadly expressed protein in human breast cancer and impacts breast cancer prognosis independently.

Although it is known that SHP2 is an important downstream effector in EGFR family signaling²³ and a study by Zhou et al. showed that SHP2 directly dephosphorylates HER2 and leads to enhanced HER2 signaling¹², we found no correlation between the SHP2 expression and HER2 amplification in this cohort. This suggests that the impact of SHP2 on human breast cancer is not dependent on HER2. We also observed a positive association between SHP2 expression and protein tyrosine phosphatase 1B (PTP1B) expression. A study by Ferrari et al. reports that SHP2 is a substrate of PTP1B, and SHP2 expression is specifically modulated by PTP1B²⁴. This may explain the association between these two protein phosphatases in our study.

Of note, SHP2 expression proved to be an independent negative prognostic factor for OS in breast cancer. To our knowledge this is a new finding as no reports have assessed the impact of SHP2 expression on prognosis in breast cancer. Multiple studies have shown that SHP2 plays an important role in cellular transformation and cell signaling^{20, 21}. Since no mutations of *PTPN11* have been identified in breast cancer to date^{6, 25}, it is likely that overexpression of SHP2 occurs downstream of other mutated proto-oncogenes⁶ such as EGFR and PDGFR α ⁷. Investigating the specific role of SHP2 in breast cancer, Aceto et al. have shown that SHP2 acts predominantly by activating the extracellular signal-regulated kinases ERK1 and ERK2⁶, which in turn leads to altered transcription of cell cycle genes and uncontrolled cell growth.

Additionally, the gene encoding the SHP2 binding protein GAB2 is frequently amplified in breast cancers^{6, 7} and can, by acting through SHP2, lead to excessive proliferation of mammary epithelial cells as well as potentiate the effect of HER2 dependent cell transformation and carcinogenesis⁹. It is thus very feasible that SHP2 expression promotes oncogenic signaling and dedifferentiation in breast cancer, contributing to a worse prognosis. Our results are consistent with the findings by Aceto et al., demonstrating that SHP2 promotes breast cancer progression and metastasis in murine models⁶ and thus provide an important link between studies in breast cancer cell lines and human breast cancer.

In subgroup analyses SHP2 expression was associated with worse OS in two of the intrinsic breast cancer subtypes (luminal A and the luminal B HER2⁻ subtype). We also observed a trend towards worse OS with SHP2 expression in two other subtypes (luminal B HER2⁺ and HER2 subtype). There were significantly more cases in the luminal A and the luminal B HER2⁻ subtypes, so the lack of statistical significance in the luminal B HER2⁺ and HER2 subtypes may be based on lack of statistical power. The basal-like subtype was the only subtype where SHP2 expression did not appear to have any effect on OS. This is significant since the study by Aceto et al. showed a fundamental role for SHP2 in triple negative breast cancers⁶. Their work however, is based on a limited number of human breast cancer cell

lines grown in mice and these cell lines may not capture the complex biology and heterogeneity of triple negative human breast cancer.

Our study is the largest TMA study to date to evaluate SHP2 expression in breast cancer, and is the first to demonstrate that SHP2 expression is an independent predictor of prognosis in breast cancer. Our data suggests that SHP2 represents a potential target in human breast cancer. This has important implications as selective small molecule inhibitors of SHP2 are currently under development^{23, 26}. Routine staining for SHP2 in breast cancer could be used to identify patients eligible for targeted therapy, but could also be used to identify those patients with a more aggressive course of disease. Additional studies are needed to define the role of SHP2 in human breast cancer.

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Figure 1.

Representative photographs of SHP2 expression in breast cancer with **a** weak cytoplasmic expression (1+) **b** moderate cytoplasmic expression (2+) **c** strong cytoplasmic expression (3+) of tumor cells. SHP2 staining, magnification 400x.

Figure 2.

Kaplan-Meier survival curve for **a** overall survival depending on SHP2 expression (univariate analysis) **b-f** Kaplan-Meier survival curve for overall survival depending on SHP2 expression for the intrinsic breast cancer subtypes.

Table 1

Basic demographic data for 1401 evaluable breast cancer cases

Mean tumor size (mm)	30.7	
Mean age at diagnosis (years)	63.5	
Tumor size	Number (n)	Percent (%)
pT1	385	27.5
pT2	737	52.6
pT3	107	7.6
pT4	172	12.3
Lymph node involvement		
pN0	725	51.8
pN1	539	38.6
pN2	134	9.6
Tumor grade		
1	323	23.0
2	576	41.1
3	502	35.9
Histologic subtype		
Invasive ductal	992	70.9
Invasive lobular	203	14.5
Mucinous	38	2.7
Apocrine	17	1.2
Cribiform	41	2.9
Papillary	19	1.4
Medullary	45	3.2
Other	44	3.2
Intrinsic subtype		
Luminal A (ER ⁺ and/or PR ⁺ , HER2 ⁻ , Ki-67 < 14%)	217	15.6
Luminal B (HER2-negative) ER ⁺ and/or PR ⁺ , HER2 ⁻ , Ki-67 ≥ 14%)	683	49.0
Luminal B (HER2-positive) (ER ⁺ and/or PR ⁺ , HER2 ⁺)	156	11.2
HER2 type (ER ⁻ or PR ⁻ , HER2 ⁺)	111	7.9
Basal-like (ER ⁻ , PR ⁻ , HER2 ⁻)	227	16.3

Table 2

Association between SHP2 expression and clinicopathological parameters

Clinicopathologic parameter	SHP2 positive		SHP2-negative		p value
Mean $\pm$ SD of tumor size (mm)	30.2 $\pm$ 16.4		31.1 $\pm$ 19.1		0.3830
Mean $\pm$ SD of age at diagnosis (years)	63.8 $\pm$ 14.1		63.2 $\pm$ 13.7		0.8113
Tumor size	(n)	(%)	(n)	(%)	0.0008
pT1	169	43.9	216	56.1	
pT2	360	48.8	377	51.1	
pT3	32	29.9	75	70.9	
pT4	90	52.3	82	47.7	
Lymph node involvement					0.0263
pN0	327	45.1	398	54.9	
pN1	245	45.4	294	54.5	
pN2	77	57.5	57	42.5	
Tumor grade					0.0269
1	134	41.5	189	58.5	
2	262	45.5	314	54.5	
3	255	50.8	247	49.2	
Estrogen receptor					0.1970
ER ⁺	474	45.62	565	54.38	
ER ⁻	176	49.58	179	50.42	
HER2					0.2207
HER2 ⁺	133	49.81	134	50.19	
HER2 ⁻	515	45.66	613	54.34	
Ki67					0.5200
1–14%	169	56.71	129	43.29	
15–23%	164	54.30	138	45.70	
24–34%	164	50.77	159	49.23	
>34%	244	53.39	213	46.61	

Table 3

Association between SHP2 expression and histologic subtype

Histologic subtype	SHP2-positive		SHP2-negative		p-value
	(n)	(%)	(n)	(%)	
Invasive ductal	469	47.3	523	52.7	0.0250
Lobular	92	45.3	111	54.7	
Mucinous	14	36.8	24	63.2	
Apocrine	8	47.1	9	52.9	
Cribiform	13	31.7	28	68.3	
Papillary	8	42.1	11	57.9	
Medullary	16	35.6	29	64.4	
Other	30	68.2	14	31.8	

Table 4

Association between SHP2 expression and breast cancer intrinsic subtype

Intrinsic subtype	SHP2-positive		SHP2-negative		p-value
	(n)	(%)	(n)	(%)	
Luminal A (ER ⁺ and/or PR ⁺ , HER2 ⁻ , Ki-67 < 14%)	98	45.2	119	54.8	0.9108
Luminal B (HER2-negative) ER ⁺ and/or PR ⁺ , HER2 ⁻ , Ki-67 14%)	307	44.9	376	55.1	
Luminal B (HER2-positive) (ER ⁺ and/or PR ⁺ , HER2 ⁺)	74	47.9	82	52.6	
HER2 type (ER ⁻ , PR ⁻ , HER2 ⁺)	59	53.1	52	46.8	
Basal-like (ER ⁻ , PR ⁻ , HER2 ⁻)	110	48.5	117	51.5	

Table 5

Univariate analyses for all cases, and by intrinsic subtype, for the effect of SHP2 expression on overall survival

SHP2 expression, all cases	Hazard Ratio (95% CI)	p-value
SHP2-positive	1.458 (1.227–1.733)	<0.0001
SHP2 expression, by intrinsic subtype		
Luminal A	1.813 (1.052–3.127)	0.0323
Luminal B (HER2 ⁻)	1.572 (1.218–2.030)	0.0005
Luminal B (HER2 ⁺)	1.346 (0.822–2.204)	0.2370
HER2 type	1.457 (0.844–2.513)	0.1765
Basal-like	1.076 (0.738–1.570)	0.7024

Table 6

Multivariate analysis for the effect of clinicopathologic parameters and SHP2 expression on overall survival

Clinicopathologic parameter	Hazard Ratio (95% CI)	p-value
Age(per 1-year)	1.037 (1.030–1.044)	<0.0001
Tumor size		
pT1 (reference)	1	
pT2	1.619 (1.247–2.101)	0.0003
pT3	2.139 (1.482–3.089)	<0.0001
pT4	2.269 (1.639–3.143)	<0.0001
Lymph node involvement		
pN1 (reference)	1	
pN1	1.420 (1.165–1.732)	0.0005
pN2	2.602 (1.969–3.440)	<0.0001
Tumor grade		
BRE grade 1 (reference)	1	
2	1.582 (1.196–2.092)	0.0013
3	2.527 (1.889–3.381)	<0.0001
Intrinsic subtypes		
Luminal A	1	
Luminal B (HER2 ⁻)	1.080 (0.796–1.465)	0.6205
Luminal B (HER2 ⁺)	1.268 (0.870–1.848)	0.2159
HER2 type	1.169 (0.774–1.764)	0.4579
Basal-like	1.941 (1.369–2.752)	0.0002
SHP2 expression, all cases*		
	1.239 (1.038–1.480)	0.0177

* Interaction between SHP2 expression and intrinsic subtypes not significant (p=0.91)