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ORIGINAL ARTICLE

Expression of *SIRT1*, *SIRT3* and *SIRT6* Genes for Predicting Survival in Triple-Negative and Hormone Receptor-Positive Subtypes of Breast Cancer

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Abstract

Triple-negative breast cancer (TNBC) is characterized by aggressive phenotype and a poorer prognosis compared to the estrogen and progesterone receptor positive, Her2 negative (ER + PR + Her2-) breast cancer. Increasing evidence suggests that sirtuins, a family of histone deacetylases, could have an important role in aggressiveness of TNBC's. The current study evaluated the potential clinical relevance of *SIRT1*, *SIRT3* and *SIRT6* gene expressions in two prognostically distinctive subtypes of breast cancer, the most aggressive TNBC and the least aggressive ER + PR + Her2- tumors. Total RNAs were isolated from 48 TNBC and 63 ER + PR + Her2- tumor samples. Relative gene expression was determined by SYBR Green RT-PCR and delta-delta Ct method, normalized to *GAPDH*. Mean gene expression of both *SIRT1* and *SIRT3* was significantly lower in the TNBC compared to ER + PR + Her2- tumors ($p = 0.0001$). Low *SIRT1* and *SIRT6* expressions associated with worse overall survival in ER + PR + Her2- patients ($p = 0.039$, $p = 0.006$, respectively), while TNBC patients with high *SIRT1* tend to have a poor prognosis ($p = 0.057$). In contrast, high expression of *SIRT3* in TNBC patients associated with higher histological grade ($p = 0.027$) and worse overall survival ($p = 0.039$). The Cox regression analysis revealed that low *SIRT1* expression could be an independent prognostic marker of poor survival in ER + PR + Her2- breast cancers (HR = 11.765, 95% CI:1.234–100, $p = 0.033$). Observed differential expression of *SIRT1*, *SIRT3* and *SIRT6* genes in TNBC and ER + PR + Her2- subtypes, with opposite effects on patients' survival, suggests context-dependent mechanisms underlying aggressiveness of breast cancer. Further investigations are necessary to evaluate sirtuins as potential biomarkers and therapeutic targets in breast cancer.

Keywords Sirtuins · Gene expression · Triple-negative breast Cancer · Hormone receptor-positive breast Cancer · Survival

Introduction

Triple-negative breast cancer (TNBC) is a subtype of breast cancer defined by the absence of estrogen and progesterone receptors, and human epidermal growth factor receptor-2 (ER-PR-Her2-). According to histological subtypes, majority of TNBC tumors are invasive ductal carcinoma of no special type (NST), while the remaining 10–25% includes lobular carcinoma NST, apocrine carcinoma, adenoid cystic carcinoma, medullary carcinoma, metaplastic carcinoma, and mixed lobular-ductal carcinoma [1]. In addition to aggressive nature and poor prognosis compared to the other breast cancer subtypes, the majority of TNBC achieve only partial response to chemotherapy [1]. As opposed to TNBC, hormone receptor-positive, Her2 negative breast cancer (ER + PR + Her2-) are relatively the least aggressive, characterized by better prognosis and survival rates [1].

Epigenetic modifications, changes in gene expression that occur without changes in the DNA sequence, impact gene expression patterns in breast cancer. Increasing evidence suggests that the essential epigenetic mechanisms such as histone acetylation and deacetylation, could have an important role in

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breast cancer aggressiveness [2]. Sirtuins (silent mating-type information regulation 2 homologs) represent class III of NAD-dependent histone deacetylases (HDAC), involved in gene expression regulation by deacetylation of histone and non-histone proteins [2]. Sirtuins are involved in cell cycle regulation, DNA repair, cell survival and apoptosis, indicating their complex roles in the mechanisms underlying cancer initiation and progression. Additionally, sirtuins have been implicated to epithelial-to-mesenchymal transition [3].

SIRT1, one of the key histone deacetylases, is a nuclear enzyme involved in increasing genomic stability, gene silencing, metabolism, and cell survival. Its ability to deacetylate histones and non-histone proteins, such as p53, p73, Rb, and NF-κB [4, 5], indicates its role in cell cycle regulation, and cancerogenesis. *SIRT1* overexpression was detected and correlated with poorer prognosis in several solid carcinomas, particularly in liver and lung carcinoma [6]. In contrast, decreased SIRT1 expression was observed in other types of cancer, such as glioblastoma, ovarian, colorectal, bladder, and prostate carcinoma [7], suggesting its potential tumor suppressive role. However, studies of *SIRT1* expression in breast cancer show inconsistent results [6, 8, 9], indicating that SIRT1 could play a contradictory role either as a tumor suppressor or as an oncogene in breast cancers.

SIRT3 is localized predominantly in mitochondria, modulating the multiple metabolic pathways as a response to metabolic and genotoxic cellular stresses [10, 11]. Previously, *SIRT3* overexpression was correlated with disease-free and overall survival in breast cancer patients [12].

SIRT6 is involved in DNA repair regulation, telomere maintenance, glucose and lipid metabolism [2]. Sirtuin-mediated repression of MYC- and HIF in cancer-associated metabolic reprogramming, indicates *SIRT6* as a potential tumor suppressor [13]. However, recent studies have reported overexpression of *SIRT6* gene in different cancer types, including prostate, non-small cell lung cancer and breast cancer [13, 14], establishing its potential oncogenic role.

A distinguishing feature of sirtuins is their potential dual role in the carcinogenesis, acting as tumor suppressors, or oncogenes depending on the tumor type, stage, and microenvironment. Previous studies have shown different expression patterns among different subtypes of cancer [7, 15], including breast cancer [16, 17]. The aim of this study is to examine the potential clinical relevance of *SIRT1*, *SIRT3* and *SIRT6* gene expressions in two prognostically distinctive subtypes of breast cancer, the most aggressive TNBC and the least aggressive ER + PR + Her2- cancers.

Material and Methods

Patients

Clinicopathological features are presented in Supplement Table 1. The study included 111 breast cancer patients, 48 with TNBC and 63 with ER + PR + Her2- tumors. All patients were females with a median age 59, range 30–79 years, who underwent surgical resection at the Institute for Oncology and Radiology, Belgrade, Serbia. All patients enrolled in this study had complete excision of the primary breast tumor. In addition, none of the patients received neoadjuvant chemotherapy prior to surgery. Tumor tissue samples were fresh-frozen and stored in liquid nitrogen in the institutional tumor bank. Informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the Institute for Oncology and Radiology, Belgrade, Serbia.

RNA Extraction and Real-Time PCR

Total RNA was extracted from fresh frozen tissue samples by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol, and subsequently used for cDNA synthesis with Tetro cDNA Synthesis Kit (Bioline, London, UK). Real-time PCR was performed on ABI 7500 Real-Time PCR (Applied Biosystems, Foster City, USA), with Maxima SYBR Green PCR Master Mix (Thermo Fisher Scientific, Massachusetts, USA). Primers used for real-time PCR were previously described [18]. All reactions were performed in triplicates, blinded to clinical data. The data were analyzed using the $2^{-\Delta\Delta Ct}$ method and the N-fold change in gene expression was normalized to endogenous control Glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical Analysis

SIRT1, *SIRT3* and *SIRT6* gene expressions in TNBC and ER + PR + Her2- tissues were compared using the Mann-Whitney U test. ROC (Receiver Operating Characteristic) and AUC (Area Under the ROC Curve) analyses were used to evaluate the mRNA expression levels as potential biomarkers in breast cancer patients. The expressions were considered as high or low using optimal cutoffs suggested by the ROC curve and the Manhattan distance method [19], or arbitrarily defined as ≥2-fold gene expression change, as previously suggested [20]. An association of fold changes in gene expressions with clinicopathological characteristics of the patients was analyzed using the Chi-square test or Fisher's exact test. Survival analysis was assessed by the Kaplan–Meier estimate and compared using the log-rank test. Cox proportional hazards analysis was used to estimate the hazard ratio (HR) for overall survival, with a 95% confidence interval (95% CI). Only variables with $p < 0.200$ in univariate analysis were included in a multivariate Cox proportional hazards model, to identify the potential independent predictors of overall survival. All statistical analyses were performed using SPSS 20.0 software (IBM Corporation, USA) and the two-sided p value <0.05 was considered statistically significant.

Results

We have observed a higher incidence of invasive lobular carcinoma in our TNBC cohort (6 lobular out of 48 TNBC patients, 12.5%) in comparison to other, much larger studies. It could be attributed to a relatively small number of eligible patients and the selection bias. According to the initial selection criteria, all patients enrolled in this study did not receive neoadjuvant chemotherapy, due to its potential impact on tumor epigenetic changes and histone modifications.

Expression of SIRT1, SIRT3, and SIRT6 Genes in TNBC and ER + PR + Her2- Breast Cancers

Gene expression of *SIRT1*, *SIRT3* and *SIRT6* were compared between two prognostically different subtypes of breast cancer, TNBC and ER + PR + Her2- tumors. Patients with TNBC had a lower gene expression of *SIRT1* and *SIRT3* genes (mean 2.017 ± SEM 0.357 and mean 1.064 ± SEM 0.122, respectively) than patients with ER + PR + Her2- (mean 12.504 ± SEM 2.202 and mean 2.330 ± SEM 0.274, respectively), with significance $p = 0.0001$, Fig. 1. ROC analysis was used to evaluate the prognostic potential of *SIRT* genes in breast cancer.

However, as previously recommended by Kim et al., less than twofold change differences in gene expression might be the effect of the imprecise nature of SYBR Green semi-quantitative RT-PCR (20). Thus, with an exception of *SIRT1* gene expression in TNBC patients where the cutoff was defined as a 2.51-fold change, according to ROC analyses (AUC 0.63, sensitivity 41.7%, specificity 83.3%), cutoffs for predicting a negative outcome were defined as ≥2-fold gene expression changes of normalized mRNA.

Association of SIRT1, SIRT3 and SIRT6 Gene Expressions with Clinicopathological Features

Associations of *SIRT1*, *SIRT3* and *SIRT6* fold changes of gene expressions with clinicopathological characteristics of TNBC and ER + PR + Her2- breast cancer patients are presented in Table 1. Low expression of *SIRT1* was associated with tumor size in ER + PR + Her2- patients ($p = 0.036$), while *SIRT3* overexpression correlated with histological ($p = 0.027$) and nuclear grade ($p = 0.050$) in TNBC patients (Table 1). In ER + PR + Her2- patients, our results showed a significant association of low *SIRT3* expression with a lobular subtype, while high *SIRT3* was more frequent in ductal subtype ($p =$ 0.047, Table 1).

Association SIRT1, SIRT3 and SIRT6 Gene Expressions with Overall Survival and Hazard Ratio

To our knowledge, all cases died from breast cancer. However, in some cases, attribution of a single cause of death may be difficult and death from a specific cause can be misattributed and be a source of bias. Thus, we have used overall survival rather than disease-specific survival, as the most reliable and available survival measure. In the overall breast cancer cohort, Kaplan-Meier analysis revealed that patients with low expression of *SIRT1* (52/111) demonstrated poorer overall survival compared with those with high expression (59/111) of *SIRT1* ($p = 0.038$, log-rank test, Fig. 2). However, stratified analysis according to breast cancer subtypes of TNBC and ER + PR + Her2-, indicated that ER + PR + Her2- patients with low *SIRT1* gene expression (47/62), had worse overall survival ($p = 0.010$, Fig. 3a), while TNBC patients with high *SIRT1* (11/48) tend to have poor overall survival ($p = 0.057$, Fig. 3b). TNBC patients with high *SIRT3* expression (6/48) had worse overall survival ($p =$ 0.039, Fig. 3c). ER + PR + Her2- breast cancer patients with low *SIRT6* expression had worse overall survival compared to the ER + PR + Her2- patients with *SIRT6* overexpression ($p =$ 0.006, Fig. 3f). *SIRT6* gene expression was not associated with overall survival overall survival in TNBC breast cancer patients.

The univariate Cox hazards regression analysis in TNBC patients revealed that the covariates tumor size (HR = 2.917,

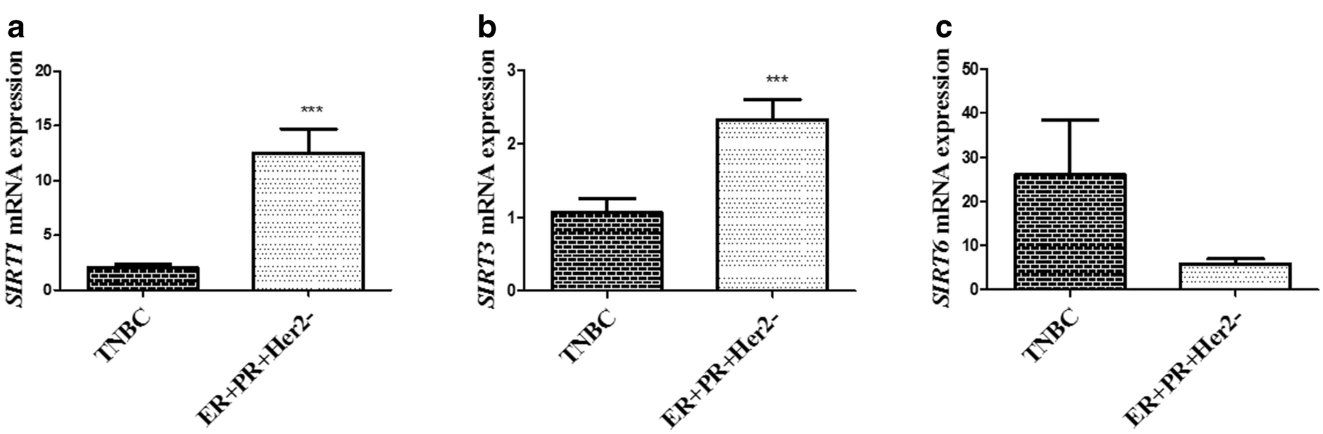

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Table 1 Association of *SIRT1, SIRT3* and *SIRT6* gene expression with clinicopathological features in TNBC and ER + PR + Her2- patients

		TNBC						ER + PR + Her2-					
Gene expression		*SIRT1*		*SIRT3*		*SIRT6*		*SIRT1*		*SIRT3*		*SIRT6*	
Clinicopathol. features		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Histol. type	**Ductal**	25	6	26	5	7	24	7	19	13	14	10	17
	Lobular	3	3	6	0	2	4	8	23	22	9	11	20
	Others	9	2	10	1	2	9	0	5	1	4	3	2
	p	*NS*		*NS*		*NS*		*NS*		*0.047**		*NS*	
Age (median)	**< 59**	16	6	19	3	7	15	9	29	25	13	14	24
	> 59	21	5	23	3	4	22	6	19	11	14	10	15
	p	*NS*		*NS*		*NS*		*NS*		*0.087*		*NS*	
Menopausal status	**premenopause**	6	0	5	1	3	3	3	18	14	7	7	14
	menopause	31	11	37	5	8	34	11	31	22	20	15	27
	p	*NS*		*NS*		*NS*		*NS*		*NS*		*NS*	
Tumor size (cm)	**<2**	11	2	10	3	2	11	4	16	12	8	9	11
	2–5	24	7	29	2	9	22	9	32	22	19	14	27
	>5	2	2	3	1	0	4	2	0	2	0	1	1
	p	*NS*		*NS*		*NS*		*0.036**		*NS*		*NS*	
Histol. grade	**hG1/2**	15	5	20	0	4	16	15	47	36	26	23	39
	hG3	22	6	22	6	7	21	0	1	0	1	1	0
	p	*NS*		*0.027**		*NS*		*NS*		*NS*		*NS*	
Nuclear grade	**nG1/2**	12	4	16	0	4	12	15	48	36	27	24	39
	nG3	23	6	23	6	6	23	0	0	0	0	0	0
	p	*NS*		*0.050*		*NS*		*NS*		*NS*		*NS*	
Nodal status	**Positive**	15	10	21	2	6	17	7	24	18	13	13	18
	Negative	18	5	21	4	5	20	8	24	18	14	11	21
	p	*NS*		*NS*		*NS*		*NS*		*NS*		*NS*	
Metastasis	**Positive**	9	3	11	1	2	10	1	7	4	4	2	6
	Negative	28	8	31	5	9	27	14	41	32	23	22	33
	p	*NS*		*NS*		*NS*		*NS*		*NS*		*NS*	

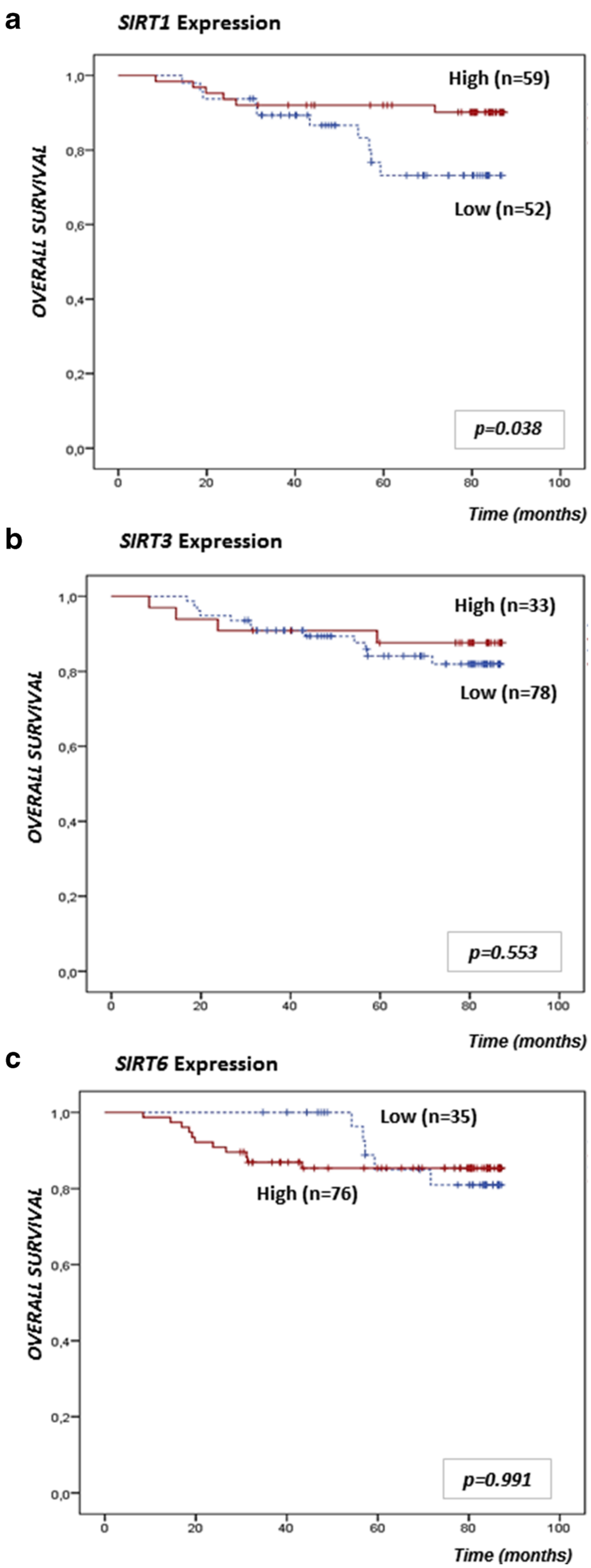
*Statistically significant data; *NS* - Non-significant data

95% CI:1.030–8.260, $p = 0.044$), metastasis (HR = 5.169, 95% CI:1.595–16.755, p = 0.006), and recurrences (HR = 7.334, 95% CI:2.133–25.217, $p = 0.002$) significantly contributed to poor survival, while *SIRT1* and *SIRT3* gene expressions showed a trend for an association with overall survival, ($p = 0.073$ and $p = 0.055$, respectively, Table 2). The univariate Cox regression analysis indicated that in ER + PR + Her2- patients with low *SIRT1* expression (HR = 11.83, 95% CI:1.23–111.94, $p = 0.033$) significantly contributed to poor survival, while the patients with metastasis had a tendency to have a worse overall survival (HR = 6.946, 95% CI:0.980–49.340, $p = 0.053$, Table 2).

Multivariate Cox regression analysis model, that included the variables with significance below 0.200, revealed that *SIRT1* expression status persisted as an independent prognostic factor for worse survival for ER + PR + Her2- patients (HR = 11.765, 95% CI:1.234–100, p = 0.033, Table 2). In TNBC subtype, multivariate analysis showed that recurrences persisted as an independent predictor of poor survival (HR = 7.334, 95% CI:2.133–25.217, $p = 0.002$, Table 2).

Discussion

The biological role of sirtuins, family of class III histone deacetylases is not fully elucidated, and their dual role in the carcinogenesis remains controversial [2]. Several studies, including ours, indicated both oncogenic and tumor-suppressive role of sirtuins in the specific tumor type, depending on the cellular context and molecular subtype. Depending on the tumor type, stage and context, sirtuins might show both the tumor promoting or tumor suppressing roles in different types of cancer [6, 7], and recent studies revealed different expression profiles of *SIRT* genes in specific subtypes of breast cancer [16]. Previous studies of association of sirtuins expressions



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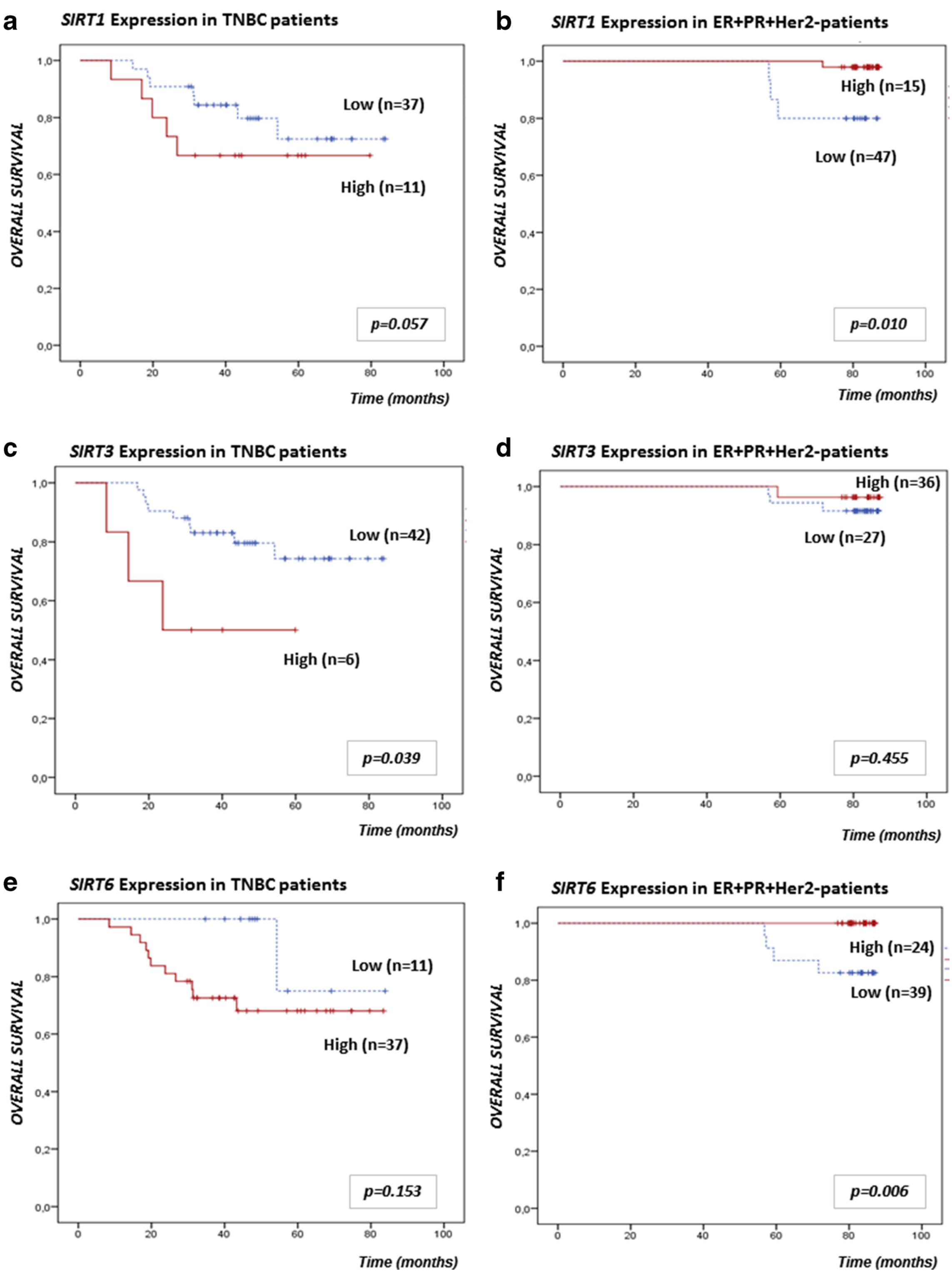
with survival in breast cancer showed controversial results [6, 8, 9, 21].

In the current study, we investigated the potential clinical relevance of *SIRT1*, *SIRT3* and *SIRT6* gene expressions in two prognostically distinctive subtypes of breast cancers, triple-negative (ER-PR-Her2-), and ER + PR + Her2- breast cancer. Our research revealed significantly lower expressions of *SIRT1* and *SIRT3* genes in TNBC compared to ER + PR + Her2- tumors. In the overall cohort, *SIRT1* under-expression correlated to poor overall survival of breast cancer patients. Stratification by breast cancer subtype revealed that low *SIRT1* expression was an independent predictor of unfavorable prognosis in patients with ER + PR + Her2- tumors, while *SIRT3* overexpression predicted worse overall survival in TNBC patients.

Our findings of lower *SIRT1* and *SIRT3* expressions in TNBC compared to ER + PR + Her2- tumors are in accordance with previous findings [16, 22]. Rifai et al. demonstrated that *SIRT1* was overexpressed in luminal A and luminal B, as well as Her2-enriched breast cancers, while significantly lower expression was observed in triple-negative subtype cancers [16]. In addition, Desuki et al. observed that lower *SIRT3* expression is more frequent in ER-negative compared to ER-positive breast cancer [22]. Our findings also point out to different expression patterns of *SIRT1* gene expression in two prognostically distinctive subtypes of breast cancer.

In the present study, low *SIRT1* expression significantly correlated with poor survival in ER + PR + Her2- subtype of breast cancer, in support of the potential tumor suppressing role of SIRT1. Our findings are in line with previous results in a number of human carcinomas, including glioma, bladder, prostate, and ovarian cancer, where the expression levels of *SIRT1* are decreased [23]. Our results of the potential tumor suppressing role of SIRT1 are in agreement with findings of SIRT1 effect on the suppression of the epithelial-mesenchymal transition (EMT) and breast cancer metastasis formation in nude mice [24], and, as well as its effect on c-MYC repression [25].

However, our results do not support the findings of several other studies indicating the potential oncogenic role of SIRT1 in luminal tumors [16, 26]. The decrease in *SIRT1* expression was in correlation with increased tumor aggressiveness and poor prognosis [16]. SIRT1 was shown to be essential for estrogen-induced breast cancer growth, where its inactivation eliminated estrogen/ERα-induced cell growth and tumor development and triggered apoptosis [26]. In ER-positive breast cancer cell lines, SIRT1 binding to ERα caused the transcriptional repression of p53 and cyclin G2, inducing the cell growth and suppressing apoptosis [26]. On the other hand, in TNBC subtype we observed an inverse pattern where patients with *SIRT1* overexpression tend to have unfavorable clinical outcomes ($p = 0.057$), indicating the potential oncogenic role of SIRT1 in TNBC subtype. In vitro experiments



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showed that SIRT1 inhibition significantly reduced cell growth, proliferation, and viability [23], indicating its oncogenic potential. Previously, *SIRT1* was associated with tumor invasion, lymph node metastasis, and poor disease-free survival in TNBC [21]. Also, inhibition of *SIRT1* expression with small interfering RNA suppressed tumor invasion in MDA-MB-231 [9], a highly aggressive and invasive TNBC cell line. In contrast, it was shown that SIRT1 could act as a tumor suppressor in triple-negative breast cancer cells, inhibiting cancer proliferation and cell growth via targeting p53 [5, 27].

Table 2 Univariate and Multivariate Cox Regression Analysis in TNBC and ER + PR + Her2- breast cancer patients

	Variables	TNBC		ER + PR + Her2-	
		HR [95% CI] [a]	*p*	HR [95% CI] [a]	*p*
UNIVARIATE ANALYSIS	**Age ≥ median (59)**	1.677 [0.505–5.572]	*0.399*	0.503 [0.052–4.840]	*0.552*
	Menopausal status	1.408 [0.180–11.016]	*0.745*	1.528 [0.159–14.688]	*0.714*
	Histol. grade	1.684 [0.507–5.601]	*0.395*	1.732 [0.028–105.608]	*0.793*
	Nuclear grade	1.104 [0.323–3.775]	*0.875*	21.666 [0–126.000]	*0.765*
	Tumor size	2.917 [1.030–8.260]	*0.044**	0.456 [0.070–2.989]	*0.413*
	Nodal status	0.511 [0.154–1.703]	*0.275*	1 [0.141–7.098]	*1*
	Metastasis	5.169 [1.595–16.755]	*0.006**	6.946[0.980-49.340]	*0.053*
	Recurrences	7.334 [2.133–25.217]	*0.002**	4.73 [0.66–33.55]	*0.121*
	***SIRT1* mRNA low expression**	0.349 [0.110–1.103]	*0.073*	11.83 [1.23–111.940]	*0.033**
	***SIRT3* mRNA high expression**	3.622 [0.972–13.501]	*0.055*	0.433 [0.045–4.162]	*0.468*
	***SIRT6* mRNA low expression**	0.251 [0.032–1.947]	*0.186*	170.189 [0.018-inf.]	*0.270*
MULTIVARIATE ANALYSIS	**Recurrences**	7.334 [2.133–25.217]	*0.002**	–	–
	***SIRT1* mRNA low expression**	–	–	11.765 [1.234–100]	*0.033**

*Statistically significant data

[a] HR indicates a hazard ratio; CI, confidence interval

Our results suggesting potential tumor-promoting role of *SIRT1* in TNBC are in line with findings of *SIRT1* overexpression associated with poor prognosis in TNBC patients [21, 28] and with those having demonstrated that *SIRT1*-siRNA suppress *SIRT1* expression and tumor invasion in TNBC cell line [9]. Also, SIRT1 promoted tumor growth both in vivo and in vitro in ER-negative breast cancer through GPER and subsequent activation of EGFR/ERK/c-fos/AP-1 signaling pathway [29]. A meta-analysis revealed that *SIRT1* was an unfavorable prognostic factor in breast cancer patients [6].

Our results demonstrated an overall significantly lower expression of *SIRT3* gene in patients with TNBC, compared to patients with ER + PR + Her2- cancer. In accordance with our findings, the study of Desuki et al. also observed that *SIRT3* under-expression is more frequent in ER-negative compared to ER-positive breast cancers [22]. However, we observed that in the TNBC group, *SIRT3* gene overexpression was associated with higher histological grade, implicating that *SIRT3* might have a role in tumor cell dedifferentiation. Likewise, in ER + PR + Her2- group *SIRT3* gene under-expression was more frequent in lobular histological type, as opposed to poorly differentiated invasive ductal carcinomas NST. Furthermore, TNBC patients with overexpression of *SIRT3* had shorter overall survival, while *SIRT3* expression did not have an impact on survival in ER + PR + Her2- cancer patients. Our results are in line with previous findings of *SIRT3* where overexpression was previously correlated with lymph node status, grade, tumor size, disease-free and overall survival in breast cancer patients [12]. Also, our results are in accordance with the recent study that indicated that *SIRT3* might have a tumor-promoting role in breast cancer [30]. However, our results are opposed to the findings of Desuki et al. who showed that low *SIRT3* expression is associated with low survival rates in all subtypes of breast cancers [22].

Previous studies reported controversial findings on *SIRT6* gene expression in breast cancer [31–33]. We observed that low *SIRT6* expression was associated with poor overall survival of ER + PR + Her2- breast cancer patients. Our results of the potential tumor suppressor role of SIRT6 in the hormone-receptor positive subtype of breast cancer are supported by the findings of Ioris et al., who demonstrated that enhanced *SIRT6* suppressed tumor proliferation and progression in vivo and in MCF7, an ER + PR+ breast cancer cell line [34]. In accordance with our results, another study showed that breast cancer patients with *SIRT6* overexpression had better overall survival compared to patients with low *SIRT6* expression [35]. However, they also observed that only non-phosphorylated *SIRT6* acted like tumor suppressor, while the phosphorylated form was associated with poor overall survival [35]. In contrast, another study revealed that overexpression of *SIRT6* increased proliferation and predicted a poor prognosis in breast carcinomas [31]. Thus, *SIRT6* could also have a dual role in breast cancer and further investigations would elucidate its potential for predicting breast cancer patient survival and the utility of *SIRT6* agonists as therapeutics.

In conclusion, our results showed different expression profiles of *SIRT1* and *SIRT3* genes in TNBC and ER + PR + Her2- tumor subtypes, with opposite effects on patients' overall survival time. Low *SIRT1* and *SIRT6* expressions were a predictor of poor survival in ER + PR + Her2- breast cancer patients, while high *SIRT3* expression correlated with worse survival of TNBC patients. Our results suggest that sirtuins could have dichotomous tumor suppressing/promoting role not only in different malignancies, but also in specific subtypes of breast cancer. The significance of *SIRT1*, *SIRT3* and *SIRT6* as predictors of survival in breast cancer remains controversial and could be context-dependent. Further investigations are needed to assess the potential clinical use of sirtuins as prognostic biomarkers, as well as therapeutic targets in breast cancer.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval In this retrospective study, all procedures were performed on already available biological material from the institutional tumor bank in accordance with the ethical standards of the institutional ethics committee (Ethics Committee of the Institute for Oncology and Radiology, Belgrade, Serbia) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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