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Balance between SIRT1 and DBC1 expression is lost in breast cancer

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SIRT1 (silent mating-type information regulation 2 homologue 1)-mediated cellular resistance to various stresses is negatively regulated by deleted in breast cancer 1 (DBC1), which was originally reported to be deleted in breast cancer. However, the suggested functions of SIRT1 as a potential tumor promoter and of DBC1 as a potential tumor suppressor have been challenged by observations of their respective down- and up-regulation in various cancers. The aim of the present study was to simultaneously evaluate the expression levels of SIRT1 and DBC1 in the normal and tumor breast tissues from 28 breast cancer patients and to determine correlations with clinicopathological variables. SIRT1 and DBC1 expression was higher in tumor tissues than in matched normal tissues at the protein level, but not at the transcriptional level. Overexpression of SIRT1 and DBC1 in tumor tissue was correlated with favorable and unfavorable clinicopathological factors, suggesting their pleiotropic functions as a potential tumor promoter and tumor suppressor in tumorigenesis. Interestingly, although the overall expression of SIRT1 and DBC1 increased in tumor breast tissues, the correlation between SIRT1 and DBC1 expression was weaker in tumor tissue than in normal tissue. This suggests that the negative regulation of SIRT1 by DBC1 may retard tumorigenesis in breast tissue. Therefore, the correlation between SIRT1 and DBC1 is a potential prognostic indicator in breast cancer. (*Cancer Sci* 2010; 101: 1738–1744)

SIRT1 is a protomember of the sirtuin family (SIRT1–7) that deacetylates histones and numerous non-histone substrates in a nicotinamide adenine dinucleotide (NAD^+)-dependent manner.[1] SIRT1 (silent mating-type information regulation 2 homologue 1) participates in a variety of physiological processes, such as gene silencing, metabolism, neuroprotection, and genomic stability.[1,2] In regards to growth of cancer cells, it has been reported that SIRT1 regulates cell proliferation, survival, and death, and plays a pivotal role in tumorigenesis.

Several studies have demonstrated a significant increase in SIRT1 in human prostate cancer,[3] ovarian cancer,[4] gastric cancer,[5] colon cancer,[6,7] breast cancer,[8,9] glioblastoma,[10] lymphoma,[11] acute myeloid leukemia,[12] and non-melanoma skin cancers.[13] On a molecular level, SIRT1 promotes cellular survival by deacetylating cell cycle regulators, including p53,[14] p73,[15] Rb,[16] and FOXOs,[17] or represses the transcription of tumor suppressors such as E-cadherin and MLH1.[18] SIRT1 inhibition sensitizes cells to ionizing radiation and chemotherapeutic reagents such as cisplatin, and reverses drug resistance.[19–21] These findings support the potential role of SIRT1 as a tumor promoter.

In contrast, SIRT1 in transgenic mice retards colon cancer growth as SIRT1 deacetylates β-catenin and promotes its cytoplasmic localization,[22] and increases overall survival in $p53^{+/-}$ mice.[23] In addition, SIRT1 induces tumor necrosis factor (TNF)-α-induced apoptosis through the deacetylation of nuclear factor-kappa B (NF-κB)[24] and is also able to negatively regulate the expression of survivin, an anti-apoptotic gene, in a breast cancer type 1 susceptibility protein (BRCA1)-dependent manner.[25] Wang *et al.*[26] reported that $SIRT1^{-/-}$ mouse embryonic fibroblasts displayed chromosome aneuploidy and repair defects. They also observed low expression of SIRT1 in breast cancer, glioblastoma, bladder carcinoma, prostate carcinoma, and various subtypes of ovarian carcinoma.[26] These findings indicate that SIRT1 may suppress tumor formation or growth. Although the precise role of SIRT1 in tumorigenesis is unclear, these findings concerning the pleiotropic nature of SIRT1 suggest that this molecule should be tightly regulated in tumorigenesis.

The expression and activity of SIRT1 are regulated at several stages, including transcription, post-transcription, and post-translational modification.[27] The activity of SIRT1 is positively regulated by active regulator of SIRT1 (AROS)[28] and negatively regulated by deleted in breast cancer 1 (DBC1).[29] Although homozygous deletion of DBC1 was initially found in a breast cancer patient,[30] microarray data have indicated an overexpression of DBC1 in breast cancer.[31] DBC1 is also thought to have both tumor suppressing and tumor promoting functions. DBC1 inhibits the deacetylase activity of SIRT1 and then enhances p53 and FOXO hyperacetylation, thereby increasing p53- and FOXO-mediated functions.[29,32] DBC1 C-terminal fragments stimulate apoptosis following treatment with TNF-α. Therefore, DBC1 may act as a tumor suppressor. In contrast, DBC1 stabilizes estrogen receptor (ER)-α[33] and acts as a coactivator of androgen receptor (AR),[34] implying that it plays a role in cancer cell survival.

Given that both SIRT1 and DBC1 are involved in cancer cell death and survival and that SIRT1 is negatively regulated by DBC1, the correlation between SIRT1 and DBC1 may be a determinant of cell fate during tumorigenesis. Therefore, we investigated the expression of SIRT1 and DBC1 in tumor breast tissues and matched normal breast tissues to determine the correlation between their expression and clinicopathological indices of breast cancer.

Materials and Methods

Patients. A total of 28 breast cancer patients were included. Tumor tissues and matched normal breast tissues were immediately frozen after collection at Kyung Hee University Medical Center in Seoul, Korea between 2004 and 2009. The cases were diagnosed as infiltrating ductal carcinomas (IDC; $n = 27$) or metaplastic carcinomas ($n = 1$), according to the World Health Organization classification system.[35] Clinical data were collected retrospectively. All patients provided informed consent and this study was approved by the Institutional Review Board of Kyung Hee Medical Center (KMC IRB 1001-02-A1).

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Antibodies. The antibodies used for western blotting were: SIRT1 (E104; Epitomics, Burlingame, CA, USA), DBC1 (antisera against 2–25 and 900–923 amino acids), and GADPH (SC-25778; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The antibodies used for immunohistochemistry (IHC) were: SIRT1 (E104; Epitomics), DBC1 (IHC-00135; Bethyl Laboratories, Montgomery, TX, USA), ER (6F11; Novocastra, Bannockburn, IL, USA), progesterone receptor (PR) (1A6; Novocastra), human epidermal growth factor receptor 2 (HER2) (A0485; Dako, Carpenteria, CA, USA), and p53 (DO-7; Dako).

Immunohistochemistry (IHC). Immunohistochemistry (IHC) for SIRT1, DBC1, ER, PR, HER2, and p53 was performed using a Bond Polymer Intense Detection System (Vision BioSystems, Melbourne, Australia) according to the manufacturer's instructions. In brief, 4-μm sections of formalin-fixed, paraffin-embedded tissues were deparaffinized by Bond Dewax Solution and immersed in Bond ER solution for 30 min at 100°C to retrieve the antigen. Endogenous peroxidase was quenched by incubation with hydrogen peroxide for 5 min at room temperature. The sections were incubated for 15 min at room temperature with primary antibodies using a Bond Intense R detection Kit (Vision BioSystems). Nuclei were counterstained with hematoxylin. One breast cancer tissue section that showed strong immunoreactivity in a pilot test was used as a positive control. As a negative control, normal horse serum was substituted for the primary antibody.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was isolated from breast tissue using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and cDNA was synthesized by SuperscriptII Reverse transcriptase (Invitrogen). The primer sequences used for PCR were as follows: SIRT1 forward 5′-CCTGTGAAAGTGATGAGGAGGATAG-3′, reverse 5′-TTGGATTCCCGCAACCTG-3′; DBC1 forward 5′-CAAACATCCCACACACTTCAC-3′, reverse 5′-GACCTGGATCCGGCTTGGATG-3′; and GAPDH forward 5′-GAGTCAACGGATTTGGTCGT–3′, reverse 5′-GGTGCTAAGCAGTTGGTGGT-3′. Synthesized cDNA was amplified and the PCR product was then visualized on 1% agarose gel. The intensity of the PCR product was determined by densitometry using the CoreBio i-MAX Gel Image Analysis System (CoreBio System, Seoul, Korea).

Western blotting (WB). Frozen tissues were homogenized and lysed with a pestle in NETN buffer (2 mM Tris–HCl [pH 8.0], 10 mM NaCl, 0.1 mM EDTA, 0.5% NP-40). Lysates were cleared by centrifugation at $14\,000 \times g$ at 4°C for 5 min. Protein was resolved by SDS-PAGE and then transferred onto a PVDF membrane (0.4 μm; Millipore, Bilerica, MA, USA). Non-specific interactions were blocked by incubating the membrane with 5% non-fat dry milk in $1 \times$ TBST for 30 min at room temperature. The membrane was then blotted with anti-SIRT1 or DBC1 as well as GAPDH antibodies at 4°C overnight. Horseradish peroxidase (HRP)-conjugated secondary antibodies were added for 2 h, and the immunoreactive signals were then detected using the Super-Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA). The level of protein expression was measured quantitatively using Image J software (version 1.41; National Institutes of Health, Bethesda, MD, USA). The SIRT1 and DBC1 signals were normalized with that of GAPDH.

Evaluation of IHC staining. All IHC slides were examined by two independent investigators (J.-Y.S. and J.L.) who were blinded to the clinical data. To compare the expression of SIRT1 and DBC1 in tumor breast tissue with that in matched normal breast tissue, the staining intensity and the staining area were evaluated semi-quantitatively. Staining intensity was quantified with a score ranging from 0 to 3. Each score was calculated according to the following equation: total score = (% cells with intensity of negative [0] × 0) + (% cells with intensity of [1+] × 1) + (% cells with intensity of [2+] × 2) + (% cells with intensity of [3+] × 3).[36] The values ranged from 0 to 300. The IHC score ratio of tumor tissue to normal tissue was used in the association analysis for clinicopathological factors. ER and PR immunoreactivity were evaluated according to the Allred scoring method.[37] Membranous HER2 staining was scored according to the HercepTest (Dako) protocol.[38] Tissue was considered p53-positive when more than 10% of the cells showed strong and distinctive nuclear immunostaining.

Breast cancer subtyping. The tumor breast samples were classified into four subtypes according to the system for the IHC subtyping of breast cancer described by Carey *et al.*,[39] with some modifications: luminal A (ER+ and/or PR+, HER2−), luminal B (ER+ and/or PR+, HER2+), HER2+/ER− (HER2+, ER−, PR−), and triple-negative (ER−, PR−, HER2−). Triple-negative samples were characterized by the absence of ER, PR, and HER2. This subtype is similar to the basal-like breast cancer subtype, which has the added characteristic of positive staining for basal cell (myoepithelial) cytokeratins (CKs) 5/6 and/or HER1.[39] Since immunostaining for CK5/6 and/or HER1 was not performed, all cases of ER−, PR− and HER2− negativity were classified as the triple-negative subtype.

Statistical analysis. The Mann–Whitney test was used to compare the mean expression of SIRT1 and DBC1 between tumor tissue and normal tissue. Fisher's exact test was used to determine the association between SIRT1 or DBC1 expression and clinicopathological factors. A P-value <0.05 was considered statistically significant. The correlation test was used to analyze correlations between SIRT1 and DBC1. All tests were two-sided.

Table 1. Characteristics of the study group (n = 28)

Characteristics	Average (range)
Age* (years)	52.4 ± 2.1 (36.0–73.0)
Tumor size* (cm)	3.0 ± 0.3 (1.0–10.0)
Disease-free survival* (months)	24.1 ± 3.5 (4.0–57.0)
Overall survival* (months)	24.8 ± 3.1 (4.0–57.0)
Characteristics	**Number of patients (%)**
ER expression	13 (46.4%)
PR expression	15 (53.6%)
Her2 expression	6 (21.4%)
pN status	
N0	13 (46.4%)
N1	8 (28.6%)
N2	3 (10.7%)
N3	4 (14.3%)
Metastasis	1 (3.6%)
TNM stage	
I	4 (14.3%)
IIa, IIb	12 (42.9%), 5 (17.9%)
IIIa, IIIb, IIIc	3 (10.7%), 0 (0.0%), 3 (10.7%)
IV	1 (3.6%)
Grade†	
1	3 (10.7%)
2	12 (42.9%)
3	13 (46.4%)
EIC	5 (17.9%)
Recurrence	3 (10.7%)
Death	3 (10.7%)

*Mean ± SE (range). †Modified Bloom–Richardson system. ER, estrogen receptor; EIC, extensive intraductal carcinoma; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Results

Patient characteristics. The characteristics of the 28 breast cancer patients are shown in Table 1. All patients were females aged between 36 and 73 years of age (52.4 ± 9.0, shown as mean ± SE). The size of tumors ranged from 1 to 10 cm (3.0 ± 0.3). Lymph node involvement was found in 15 patients, but metastasis occurred only in one patient. The TNM staging of the tumors ranged from stage I to stage IV: stage I (n = 4), stage IIa (n = 12), stage IIb (n = 5), stage IIIa (n = 3), stage IIIc (n = 3), and stage IV (n = 1). All tumors were graded according to the modified Bloom–Richardson system[40]: grade 1 (n = 3), grade 2 (n = 12), and grade 3 (n = 13). ER, PR, and HER2 were positive in 13, 15, and 6 patients, respectively. Disease-free survival and mean overall survival were 24.1 ± 3.5 months (range, 4–57 months) and 24.8 ± 3.1 months (range, 4–57 months), respectively. Three patients had recurrent disease, and three patients died. The breast cancer subtypes in the present study included luminal A (n = 14, 50%), luminal B (n = 1, 3.6%), HER2+/ER− (n = 5, 17.9%), and triple-negative (n = 8, 28.5%) subtypes.

Expression of SIRT1 and DBC1 increases in tumor breast tissues. Immunohistochemistry (IHC) revealed that both SIRT1 and DBC1 were localized to the nuclei of tumor cells and normal cells with varying intensities (Fig. 1). SIRT1 and DBC1 expression were also observed in some lymphocytes, fibroblasts, and endothelial cells of small vessels. The overall expression score of SIRT1 was 101.13 ± 14.14 (shown as mean ± SE) in tumor tissue and 29.73 ± 4.00 in normal tissue. The overall expression score of DBC1 was 151.25 ± 12.70 in tumor tissue and 88.84 ± 12.72 in normal tissue (Fig. 2a,b). The expression of SIRT1 and DBC1 was significantly higher in tumor tissue than in normal tissue ($P < 0.001$ for SIRT1; $P = 0.003$ for DBC1). There were 23 (82.1%) tumor cases with increased SIRT1 expression and 25 (89.3%) tumor cases with increased DBC1 expression. Western blotting (WB) also revealed that the overall expression of SIRT1 and DBC1 was higher in tumor tissues than in normal tissues ($P = 0.015$ for SIRT1; $P < 0.001$ for DBC1) (Fig. 2c,d). SIRT1 expression was scored as 18.61 ± 16.20 in tumor tissues, it was while scored as 13.12 ± 11.56 in normal tissues. DBC1 expression was scored as 16.47 ± 7.84 in tumor tissues and 9.79 ± 6.59 in normal tissues. There were 16 (57.1%) and 23 (82.1%) cases with up-regulated SIRT1 and DBC1, respectively. Next, to determine whether SIRT1 and DBC1 were overexpressed at the transcriptional level or the post-transcriptional level in breast tumor tissues, their mRNA levels were evaluated using RT-PCR. The expression of SIRT1 and DBC1 was not up-regulated at the transcriptional level (Fig. 2e,f). Taken together, the levels of both SIRT1 and DBC1 proteins are significantly higher in the tumor cells than in the normal cells of the breast tissues.

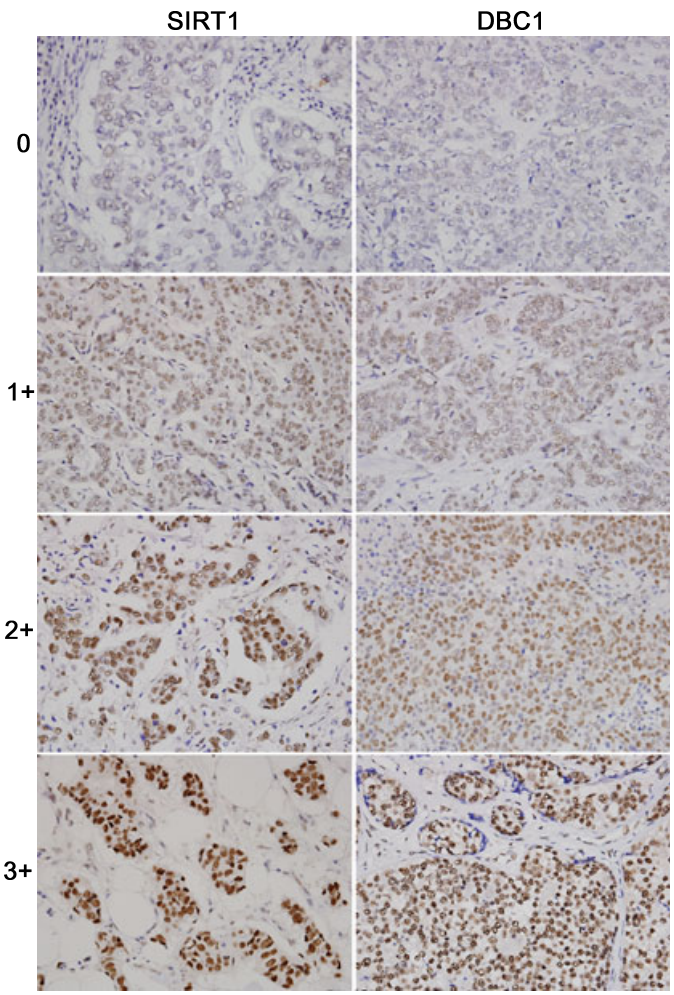

Fig. 1. Immunohistochemical scoring of SIRT1 and DBC1 expression. Left column, SIRT1 staining intensity from negative to 3+ (top to bottom); right column, DBC1 staining intensity from negative to 3+ (top to bottom) (× 400).

Clinicopathological factors are associated with overexpression of SIRT1 and DBC1 in tumor tissue. Associations between clinicopathological factors and the ratio of SIRT1 or DBC1 in tumor tissue to normal tissue are shown in Table 2. Overexpression of SIRT1 in breast tumor tissue, as detected by IHC, associated with three indices: luminal subtype (luminal A and B) ($P = 0.013$), ER expression ($P = 0.044$), and PR expression ($P = 0.013$). Up-regulation of SIRT1 in breast tumor tissue, as detected by WB, was significantly associated with lymph node metastasis ($P = 0.002$), advanced TNM stage ($P = 0.006$), low grade determined by the modified Bloom–Richardson system ($P = 0.020$), lymphovascular invasion ($P = 0.020$), and ER expression ($P = 0.009$). There was a marginal correlation between the up-regulation of SIRT1 in tumor tissue and p53 loss in tumor tissue ($P = 0.050$). In particular, ER expression appeared to significantly correlate with the overexpression of SIRT1, as determined by both IHC and WB. IHC demonstrated no significant correlations with the overexpression of DBC1 in tumor tissue. However, WB showed a significant correlation of negative p53 expression with overexpression of DBC1 ($P = 0.041$). These results indicate a stronger association for the overexpression of SIRT1 with both favorable and unfavorable clinicopathological indices than for the overexpression of DBC1.

Correlation between SIRT1 and DBC1 is lower in tumor tissue than in normal tissue. Although SIRT1 overexpression associated with several clinicopathological factors, DBC1 overexpression appeared to associate only with p53 loss. However, given that DBC1 is a negative regulator of SIRT1 deacetylase activity, we hypothesized that the correlation between SIRT1 and DBC1 has a closer association with tumorigenesis than the individual expression of either SIRT1 or DBC1, which are both up-regulated in breast tumors. Therefore, we sought to determine the correlation coefficient between SIRT1 and DBC1 in both normal tissue and tumor tissue. Surprisingly, SIRT1 and DBC1 expression showed a poor linear relationship in tumor tissue (IHC, $r = 0.137$; WB, $r = 0.040$), whereas there was a moderately strong linear relationship in normal tissue (IHC, $r = 0.542$; WB, $r = 0.335$) (Fig. 3). The finding of a lower correlation between SIRT1 and DBC1 in tumor tissue

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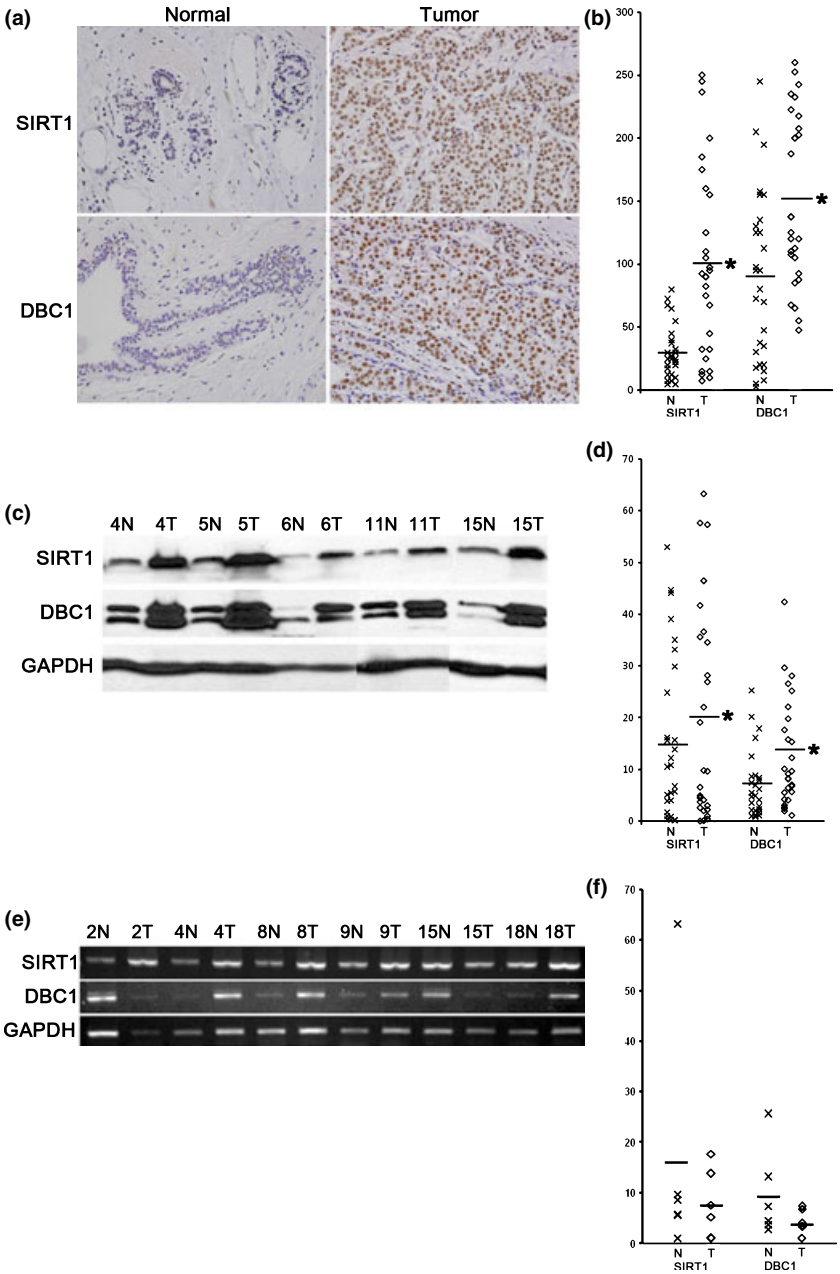

Fig. 2. Expression of both SIRT1 and DBC1, as detected by immunohistochemistry and western blotting, was higher in tumor tissues than in normal tissues. (a) SIRT1 and DBC1 expression, as demonstrated by immunohistochemistry, in normal and tumor tissue of a representative case (case no. 15) (× 400), (b) Immunohistochemistry scores of SIRT1 and DBC1 in normal and tumor tissue are shown as a scatter plot. The mean score of each group is shown as a bar, (c) The expressions of SIRT1, DBC1, and GAPDH (cases 4–6, 11, and 15) were determined by western blotting. GAPDH was used as a loading control for the analysis, (d) The densities of SIRT1 and DBC1 bands in normal and tumor tissue according to western blotting were normalized by GAPDH and are shown as a scatter plot. Means of densities for each group are shown as a bar. $*P < 0.05$, (e) The mRNA expressions of SIRT1, DBC1, and GAPDH were determined in six patients using RT-PCR, and (f) The mRNA expressions were normalized by GAPDH and the relative levels are shown as a scatter plot.

suggests that DBC1 loses its ability to inhibit SIRT1, which may be hyperactive in a tumorigenic environment.

Discussion

In the present study, we evaluated the expression of both SIRT1 and DBC1 using both immunohistochemistry and western blotting to complement the shortcomings of each individual assay. Associations between clinicopathological variables and the expression of SIRT1 and DBC1 in breast cancer tissue were investigated. The main findings are that: (i) both SIRT1 and DBC1 were overexpressed in tumor tissue; (ii) SIRT1 overexpression in tumor tissue was significantly associated with lymph node metastasis, advanced TNM stage, low grade of modified Bloom–Richardson system, luminal subtype, lymphovascular invasion, and ER and PR expression, and it was marginally associated with p53 loss; (iii) DBC1 overexpression in tumor tissue significantly correlated with p53 loss; and (iv) SIRT1 and DBC1 showed a poor linear correlation in tumor tissue, but a moderately strong linear correlation in normal tissue.

There are contrasting reports regarding the expression of SIRT1 in breast cancer tissues. Kuzmichev *et al.* demonstrated an overexpression of SIRT1, while Wang *et al.* reported a decrease in SIRT1 expression in breast tumor tissue.[8,26] However, given that SIRT1 may act as either a tumor promoter or a tumor suppressor, SIRT1 expression *per se* may not directly correlate with its role in tumorigenesis. Clinical studies have demonstrated that SIRT1 is a prognostic factor that correlates with short overall survival and unfavorable clinicopathological factors in diffuse large B-cell lymphoma and gastric cancer.[5,11] In the present study, the up-regulation of SIRT1 associated with several unfavorable clinicopathological factors such as lymph node metastasis, advanced TNM stage, and lymphovascular invasion in breast cancer. These results support a strong correlation between SIRT1 overexpression and tumorigenesis. In contrast, Jang *et al.*[4] reported that high SIRT1 expression correlated with longer overall survival in serous ovarian cancers.

Table 2. Correlations between clinicopathological variables and the expression of SIRT1 and DBC1

Characteristics	No. of patients	Immunohistochemistry				Western blotting			
		SIRT1 (T > N)*		DBC1 (T > N)*		SIRT1 (T > N)*		DBC1 (T > N)*	
	28	23 (82.1)	*P*-value†	25 (89.3)	*P*-value†	16 (57.1)	*P*-value†	23 (82.1)	*P*-value†
Age (years)									
≤52	17	15 (88.2)	0.353	15 (88.2)	1.000	10 (58.8)	1.000	14 (82.4)	1.000
>52	11	8 (72.7)		10 (90.9)		6 (54.5)		9 (81.8)	
Mass size (cm)									
≤2	8	8 (100.0)	0.281	8 (100.0)	0.536	5 (62.5)	1.000	8 (100.0)	0.281
>2	20	15 (75.0)		17 (85.0)		11 (55.0)		15 (75.0)	
LN mets									
Absent	13	9 (69.2)	0.153	11 (84.6)	0.583	3 (23.1)	**0.002**	9 (69.2)	0.153
Present	15	14 (93.3)		14 (93.3)		13 (86.7)		14 (93.3)	
Distant mets									
Absent	27	22 (81.5)	1.000	24 (88.9)	1.000	15 (55.6)	1.000	22 (81.5)	1.000
Present	1	1 (100.0)		1 (100.0)		1 (100.0)		1 (100.0)	
TNM stage									
I–IIa	14	10 (71.4)	0.326	12 (85.7)	1.000	4 (28.6)	**0.006**	10 (71.4)	0.326
IIb–IV	14	13 (92.9)		13 (92.9)		12 (85.7)		13 (92.9)	
Grade‡									
1–2	15	14 (93.3)	0.153	13 (86.7)	1.000	12 (80.0)	**0.020**	13 (86.7)	0.639
3	13	9 (69.2)		12 (92.3)		4 (30.8)		10 (76.9)	
Subtype§									
Luminal	15	15 (100.0)	**0.013**	13 (86.7)	1.000	11 (73.3)	0.125	12 (80.0)	1.000
Non-luminal	13	8 (61.5)		12 (92.3)		5 (38.5)		11 (84.6)	
LVI									
Absent	13	9 (69.2)	0.153	11 (84.6)	0.583	4 (30.8)	**0.020**	9 (69.2)	0.153
Present	15	14 (93.3)		14 (93.3)		12 (80.0)		14 (93.3)	
Death									
No	25	20 (80.0)	1.000	22 (88.0)	1.000	14 (56.0)	1.000	20 (80.0)	1.000
Yes	3	3 (100.0)		3 (100.0)		2 (66.7)		3 (100.0)	
ER									
negative	15	10 (66.7)	**0.044**	13 (86.7)	1.000	5 (33.3)	**0.009**	11 (73.3)	0.333
positive	13	13 (100.0)		12 (92.3)		11 (84.6)		12 (92.3)	
PR									
negative	13	8 (61.5)	**0.013**	12 (92.3)	1.000	5 (38.5)	0.125	11 (84.6)	1.000
positive	15	15 (100.0)		13 (86.7)		11 (73.3)		12 (80.0)	
HER2									
negative	22	18 (81.8)	1.000	19 (86.4)	1.000	13 (59.1)	1.000	17 (77.3)	0.553
positive	6	5 (83.3)		6 (100.0)		3 (50.0)		6 (100.0)	
p53									
negative	18	16 (88.9)	0.315	17 (94.4)	0.284	13 (72.2)	0.050	17 (94.4)	**0.041**
positive	10	7 (70.0)		8 (80.0)		3 (30.0)		6 (60.0)	

Data are shown as number of patients (%). *T > N, overexpression in tumor tissue of SIRT1 or DBC1. †*P*-values were determined by Fisher's exact test. Bold, statistically significant. ‡Grade, modified Bloom–Richardson system. §Luminal (luminal A and luminal B subtypes), non-luminal (HER2+/ER− and triple-negative subtypes). ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; LVI, Lymphovascular invasion; mets, metastasis; PR, progesterone receptor.

We also observed that SIRT1 up-regulation also associated with certain favorable prognostic factors, including low grade cancer, luminal subtype, and ER and PR expression. These findings suggest that SIRT1 is a multifaceted protein that potentially promotes or suppresses tumorigenesis. However, the pleiotropic functions of SIRT1 in tumorigenesis are poorly understood. SIRT1 may function as a tumor suppressor by being up-regulated against proliferation during an early stage of tumorigenesis. In contrast, the up-regulated SIRT1 may function as a tumor promoter to inhibit apoptosis and promote cell survival, and thus facilitate tumor growth. Finally, SIRT1 function may be dependent on the cellular environments. In the present study, SIRT1 overexpression significantly associated with luminal type tumors, including the luminal A (ER+ and/or PR+, HER2−) and luminal B (ER+ and/or PR+, HER2+) carcinomas. SIRT1 overexpression in luminal carcinoma patients also significantly associated with lymph node metastasis ($P = 0.033$, data not shown). Therefore, although the luminal subtype generally represents a good prognosis,[39] luminal breast cancer with SIRT1 overexpression may be more aggressive than luminal breast cancer without SIRT1 overexpression.

Similar to the fact that the precise role of SIRT1 in tumorigenesis still needs to be determined, little is known as to how DBC1, a negative regulator of SIRT1, has the opposite effects on the survival or death of cancer cells.[29,41] It has been shown that overexpression of DBC1 is an independent prognostic factor in gastric cancer, which correlates with a shorter overall survival and significantly associates with unfavorable prognostic factors, such as lymph node metastasis, advanced TNM stage, and tumor invasion.[5] In the present study, DBC1 expression also significantly increased in breast tumor tissue although overexpression only associated with loss of p53 expression, which is considered

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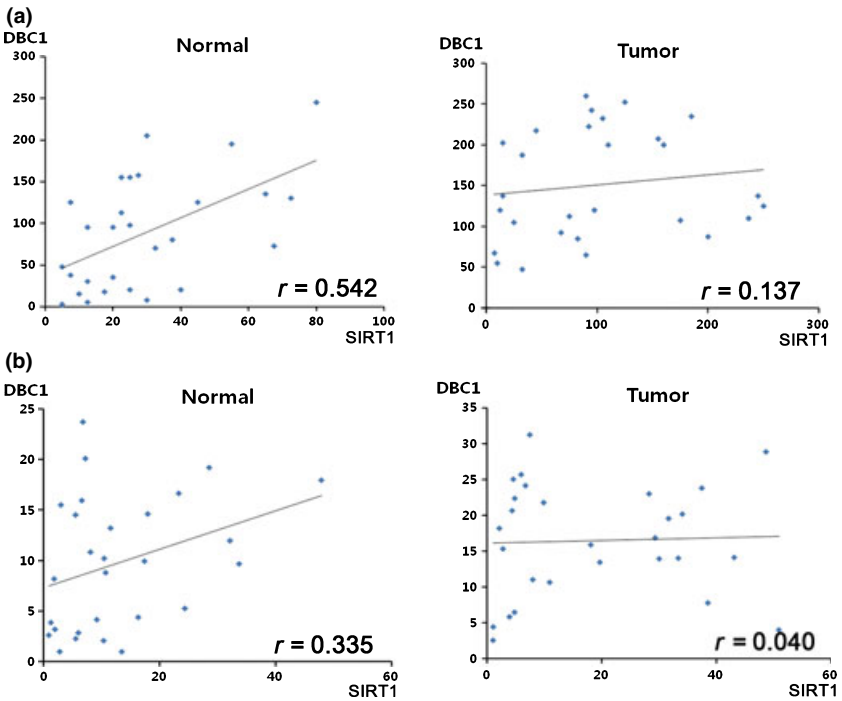

Fig. 3. Correlation between SIRT1 and DBC1 is poor in tumor tissue compared to normal tissue. The correlative value was determined by immunohistochemistry (a) or by western blotting (b).

an unfavorable prognostic factor.[42] As to why the overall expression of SIRT1 and DBC1 simultaneously increases in breast tumor tissues is an intriguing question. Since SIRT1 and DBC1 are pleiotropic proteins for tumor promotion and tumor suppression, their individual expressions are not sufficient to determine the fate of tumorigenic cells. The fact that SIRT1 deacetylase activity is inhibited by DBC1 prompted us to question as to whether the correlation between SIRT1 and DBC1 is a more direct determinant of breast cancer. Surprisingly, the correlation between SIRT1 and DBC1 was lost in tumor tissue, whereas a positive moderate correlation was observed in normal tissue. This finding suggests a new model whereby a weak correlation between SIRT1 and DBC1 contributes to tumorigenesis in breast tissue. DBC1 expression may increase in response to SIRT1 up-regulation to compensate for the hyperactivity of SIRT1, but insufficient compensation of DBC1 expression may promote tumorigenesis. This regulatory compensation may occur at the protein level, not at the transcriptional level. This model is consistent with a recent report demonstrating that high expression of SIRT1 and low expression of DBC1 lead to p53 deacetylation, which may be important in the pathogenesis of lung squamous cell carcinomas.[43] Finally, failure to counteract SIRT1 activity would interrupt SIRT1 homeostasis. However, beyond the balance between SIRT1 and DBC1, a more important determinant of tumorigenesis may be the interaction between these two proteins. Although SIRT1 interacts strongly with DBC1 *in vivo* and *in vitro*, this interaction is lost in some breast cancer cell lines.[44] Therefore, it is worthwhile to further investigate the interaction between SIRT1 and DBC1 and its effect on carcinogenesis in specific tumorigenic environments.

Our study indicates that the correlation between SIRT1 and DBC1, rather than their individual expressions, is a more useful prognostic factor in breast cancer. Therefore, the regulation of expression or interaction is a potential therapeutic target for breast cancer patients.

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Disclosure Statement

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