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The overexpression of DBC1 in esophageal squamous cell carcinoma correlates with poor prognosis

Seok-Hyung Kim[1], Jeong Hoon Kim[2,3], Eun Ji Yu[2,3], Keun-Woo Lee[2] and Cheol-Keun Park[1]

[1]Department of Pathology, [2]Samsung Biomedical Research Institute, Samsung Medical Center and
[3]Department of Health Sciences and Technology, Sungkyunkwan University School of Medicine, Seoul, Korea
*SHK and JHK contributed equally to this work

Summary. DBC1 (deleted in breast cancer 1) is a novel transcriptional coactivator that has been suggested to be a critical regulator of tumorigenesis. Recently, the overexpression of DBC1 in cancer cells has been reported to be strongly related with unfavorable clinical outcome in several cancers, including breast and gastric cancer. Despite the increasing significance of DBC1 in cancer, the expression of DBC1 and its clinical significance in esophageal squamous cell carcinoma (ESCC) have not been studied. In this study we aimed to investigate the role of DBC1 in ESCC. To this aim, we examined DBC1 expression in a total of 199 (165 ESCC and 34 normal esophageal epithelial) tissues by immunohistochemistry and assessed its prognostic value and correlation with patient survival. In addition, we measured DBC1 expression in three ESCC cell lines (TE1, TE8, and TE10). Also, we induced the loss of DBC1 expression by siRNA transfection and determined its effect on the migratory and invasive ability of cancer cells. DBC1 was expressed in all normal esophageal and ESCC tissues, whereas high expression was more prevalent in ESCC (90/165, 54.5%) than in normal esophageal (1/34, 2.8%) epithelium ($P<0.001$). Furthermore, DBC1 expression was significantly associated with poor prognosis in both univariate (relative ratio=2.889, $P<0.001$) and multivariate (relative ratio=2.655, $P<0.001$) analyses. DBC1 was also upregulated in all three ESCC cell lines, and the loss of DBC1 led to a significant reduction in the migration and invasion of tumor cells.

Our study suggests that DBC1 may promote tumor progression, and DBC1 could be a prognostic biomarker in ESCC.

Offprint requests to: Cheol-Keun Park, MD., Ph.D., Department of Pathology, Samsung Medical Center, Sungkyunkwan University, School of Medicine, 50 Ilwon-dong, Gangnam-gu, Seoul 135-710, Korea. e-mail: cheolkeun.park@samsung.com

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Introduction

Esophageal cancer is a highly aggressive neoplasm and is the eighth most common cancer and the sixth leading cause of cancer death, with 13,000 deaths per year worldwide (Yuen et al., 2007). Whereas the incidence of esophageal adenocarcinoma in western countries is increasing, the predominant type of esophageal cancer is still squamous cell carcinoma (Lam, 2000). Although diagnostic and surgical techniques for esophageal cancer have improved over the past decade, survival rates have not. The 5-year survival rate of patients with surgically treated esophageal cancer is about 30%, despite combined chemotherapy and radiotherapy (Adham et al., 2000; Ando et al., 2000). The postoperative prognosis does not always depend on the pathologic stage. Therefore, reliable and independent prognostic markers for further therapies are needed.

Deleted in breast cancer 1 (DBC1, KIAA1967, p30 DBC) is a novel transcriptional coactivator which binds to transcription factors and mediates the function of transcription factors (Kim et al., 2009). Originally, the gene encoding DBC1 was initially identified during a representative differential analysis to search for candidate breast tumor-suppressor genes on the human chromosome 8p21 region, which is frequently deleted in breast cancers (Hamaguchi et al., 2002). However, the significance of DBC1 in cancer is still controversial (Kim et al., 2009). Because DBC1 is a transcriptional coactivator that supports transcription factor, DBC1 itself is not able to directly regulate the expression of specific genes. Therefore, the effect of DBC1 on cellular behavior is influenced by transcription factors that

associate with DBC1. In this context, DBC1 may function in cancer as either a tumor promoter or a tumor suppressor depending on the cellular context. On one hand a series of studies suggest that DBC1 may function as a tumor suppressor gene. According to Sundararajan et al. (Sundararajan et al., 2005), DBC1 enhances TNFα induced apoptosis after cleavage and cytoplasmic translocation during TNFα induced apoptosis. Furthermore, DBC1 was reported to inhibit SIRT1, which generally promotes tumorigenesis by inactivating FOXO, TP53, etc. (Kim et al., 2008; Zhao et al., 2008). In addition, frequent silencing of DBC1 has been reported in non-small cell lung cancer (Izumi et al., 2005), and chromosomal loss of 8p containing DBC1 has been reported in sporadic colon cancer (Reid et al., 2009). However, increasing evidence strongly suggests that DBC1 may promote tumor progression. Hiraike et al. (Hiraike et al., 2010) reported that DBC1 is a transcriptional repressor for BRCA1, the major tumor suppressor gene in breast cancer. DBC1 was also documented to inhibit another tumor suppressor gene, SUV39H1 methyltransferase, which mediates the trimethylation of histon H3 lysine 9 (Li et al., 2009). Furthermore, DBC1 was reported to be associated with poor prognosis for gastric and breast cancer (Cha et al., 2009; Lee et al., 2011).

However, in spite of the increasing significance of DBC1 in cancer, the expression and clinical relevance of DBC1 in ESCC has not been reported. In this study, we examined the expression rate of DBC1 in ESCC and its prognostic impact on the survival of patients with ESCC. In addition, we also assessed DBC1 expression in ESCC cell lines and examined the effect of DBC1 deletion on the function of cancer cells.

Materials and methods

Patients, tissue samples, and reagents

We investigated 199 cases of normal esophagus and esophageal squamous cell carcinoma. The data were procured from surgical pathology files kept at the Pathology Department of Seoul Samsung Medical Center, Korea: 165 cases of ESCC and 34 cases of normal esophageal tissues. The pathologic feature of specimens was classified based on the 6th edition of the TNM classification (UICC). The same chemotherapy regimen was applied and all chemotherapy was conducted after surgery. All archival materials were routinely fixed in 10% neutral-buffered formalin and embedded in paraffin. The immunostaining kits were purchased from DAKO Inc. (Glostrup, Denmark).

Preparation of tissue microarray

To prepare the tissue microarray, we punched out tissue columns (2.0 mm in diameter) from the original blocks and inserted them into new paraffin blocks (each containing 47 holes to accommodate the above mentioned tissue columns).

Immunohistochemical staining procedure

Immunostaining for DBC1 protein was performed using rabbit polyclonal antibody recognizing DBC1 specifically (A300-432A, Bethyl Laboratories, Montgomery, TX, USA). Tissue sections embedded in the microslides were deparaffinized with xylene, hydrated in serial dilutions of alcohol, and immersed in peroxidase-blocking solution (Dako REAL™ Peroxidase-Blocking Solution, DAKO) to quench endogenous peroxidase activity. The sections were then microwaved in 10mM Tris buffer (pH 9.0) for 15 minutes. The sections were then incubated with the primary antibody (dilution ratio, 1:1500) for 3 hours and rinsed three times successively with washing buffer. Further incubation was performed using the DAKO REAL™ EnVision™/HRP, Rabbit/Mouse (Envision) detection reagent for 1 hour at room temperature.

Evaluation of results of the immunohistochemical staining

In this study, we used the scoring method of Sinicrope et al. (1995) to evaluate both the intensity of immunohistochemical staining and the proportion of stained epithelial cells. The staining intensity was further classified as follows: 1 (weak), 2 (moderate), and 3 (strong). The positive cells were quantified as a percentage of the total number of epithelial cells and were assigned to one of the following five categories: 0 (<5%), 1 (5%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (>75%). The percentage of positivity of the tumor cells and the staining intensities were then multiplied to generate an immunohistochemistry (IHC) score for each tumor specimen. An IHC score between 1 and 7 is considered to indicate "low or moderate expression" and an IHC score between 8 and 12 is considered to indicate "high expression" Each lesion was separately examined and scored by two pathologists (S.H.K and C.K.P). The pathologists discussed any cases showing a discrepancy in scores until a consensus was reached.

Western blotting

Cells were lysed in the presence of 50 mM Tris (pH 7.5), 150 mM NaCl, and 0.5% NP-40 on ice. Fifty micrograms of total protein from each sample was separated by using a 4%-12% polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The blots were then probed with anti-DBC1 antibody (A300-432A, Bethyl Laboratories).

RNA extraction

Cell pellets were dissolved in 1 ml of TRIZOL

(Invitrogen, Carlsbad, CA, USA) and homogenized by passing through a syringe. Then, 0.2 ml of chloroform was added and the samples were vigorously vortexed. After the solution was centrifuged at 12,000 x g for 15 minutes at 4°C, the aqueous phase was collected and then RNA was obtained by ethanol precipitation.

cDNA synthesis and evaluation of RNA by real-time RT-PCR

cDNA was synthesized from 2 μg of total RNA using Superscript II RNA Reverse Transcriptase (no. 11904-018, Invitrogen) according to the manufacturer's protocol.

Real-time RT-PCR was performed using an ABI 7900 HT Fast Real Time PCR system (Applied Biosystems, Foster City, CA, USA). PCR reactions were prepared in a final volume of 10 μL, with 5 μL of 2X Power Sybrgreen PCR master mix (Applied Biosystems), 1 μL of cDNA, and 0.25 μL each of the 10 pmol/μL forward and reverse primers. Thermal cycling conditions consisted of DNA polymerase activation at 95°C for 10 minutes, 40 cycles of denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute. Each measurement was performed in duplicate or triplicate. The threshold cycle (Ct), the fractional cycle number at which the amount of amplified target reached a fixed threshold, was determined. For all RT-PCR analyses GAPDH mRNA was used to normalize RNA inputs.

The primers were as follows: DBC1: forward primer (5'-TCAGGCACAGCTTCAACATC -3') and reverse primer (5'- GTGAAGACCCGCTGTTTCTC-3') GAPDH: forward primer (5'- GCACCGTCAAGGCT GAGAA-3') and reverse primer (5'- AGCATCGCCC CACTTGATT-3')

Cell culture

Human esophageal squamous carcinoma TE1, TE8, and TE10 cell lines were purchased from RIKEN (Saitama, JAPAN). These cells were maintained in RPMI medium supplemented with 10% fetal bovine serum.

RNAi and transient transfection

The ablation of DBC1 was performed by transfection of TE10 cells with small interfering RNA (siRNA) duplex oligos synthesized by SamCheonLee Ltd (Seoul, Korea). Control siRNA (Scrambled RNA, Genolution, Seoul, Korea) and DBC1-specific siRNA ((sense): 5'-CAG CGG GUC UUC ACU GGU A(dTdT)-3', (antisense) : 5'-UAC CAG UGA AGA CCC GCU G(dTdT)-3') which covered mRNA regions of nucleotides 1379-1397 (amino acids 460-466) of DBC1 were transfected into TE10 cells using G-Fectin reagent (Genolution, Seoul, Korea). Three days after transfection, the cells were collected for *in vitro* migration and invasion assays.

Migration and invasion assay

In vitro Matrigel invasion assays were done using 6.5 mm Costar transwell chambers (8 Am pore size; Corning, NY, USA). The Transwell filters were coated with appropriate Matrigel (1 mg/ml) (Becton Dickinson, Franklin Lakes, NJ, USA). After the Matrigel solidified at 37°C, $1x10^5$ cells were seeded onto the Matrigel. After incubation for 22 hours, the filter was gently removed from the chamber and the noninvasive cells on the upper surface were removed by wiping with a cotton swab. The cells that invaded the Matrigel and attached to the lower surface of the filter were fixed with methanol and stained with H&E solution. The number of cells attached to the lower surface of the polycarbonate filter was counted at x400 magnification under a light microscope. The migration assay was conducted in the same way as the invasion assay, except for coating with matrigel. Each type of cell was assayed in triplicate.

MTT and colony formation assays

TE10 cells transfected with siNS or siDBC1 were plated at a density of $2x10^4$ cells/well in 24 well plates and grown for 5 days. Cell proliferation was determined by MTT assays (Promega, Fitchburg, WI, USA). For colony forming assays, TE10 cells transfected with siNS or siDBC1 ($1x10^3$ cells/well) were plated in 6-well plates. After 10 days, cells were fixed with 60% methanol for 20 min and then stained with 0.1% crystal violet for 10 min. Bound dye was eluted with 10% SDS and quantified at 570 nm.

Statistical analysis

Statistical analyses were conducted using Pearson's correlation coefficient, Fisher's exact tests, Pearson's χ^2 tests, ANOVA, Mann-Whitney tests, Tukey's HSD, and Duncan's test (as a *post hoc* test). *P* values less than 0.05

Table 1. Results of the immunohistochemical analysis of DBC1 expression in normal and ESCC tissues.

	No expression	Low to moderate expression	High expression
Squamous cell carcinoma (n=165)	0 (0.0%)	75 (45.5%)	90 (54.5%)
Normal (n=34)	0 (0.0%)	33 (97.1%)	1 (2.9%)

($p<0.001$)

were regarded as statistically significant. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

DBC1 protein expression in normal esophagus and esophageal squamous cell carcinoma

The immunohistochemical analysis revealed that DBC1 expression was frequent in normal esophageal and ESCC tissues. Most of the DBC1 staining was nuclear, and cytoplasmic staining of DBC1 was rarely observed (Fig. 1). Therefore, we evaluated nuclear DBC1 expression only. Of the 165 ESCC specimens examined in this study, 90 (54.5%) were strongly positive for DBC1 immunoreactivity (Table 1). In normal esophageal tissue, DBC1 expression was also prevalent, although high expression was rare (1/34, 2.9%). As a result, there was a significant difference in the rate of DBC overexpression between normal and cancerous esophageal epithelium (p<0.001, Table 1). The association between DBC1 expression and clinicopathologic variables is shown in Table 1. DBC1 overexpression was more frequent in poorly differentiated cancer (p=0.039, Table 2). However, there was no significant correlation between DBC1 expression and patient age, sex, lymph node metastasis, tumor size, or TNM stage (Table 2).

Association of DBC1 over expression with shorter survival time in ESCC patients

Results of the univariate Cox proportional hazard analysis of the relation between each clinicopathologic variable and relapse-free survival and overall survival are shown in Table 3. Poor differentiation of cancer cells, a high TNM stage and the presence of lymph node metastasis predicted shorter overall survival and relapse-free survival (Table 3). Overexpression of DBC1 was

Table 2. Relation between the expression of DBC1 and clinicopathologic variables.

	Case no. (n=165)	DBC1 staining		*P* value
		Low to moderate expression (%)	High expression (%)	
Age				
≥65 years	127	59(46.5)	68(53.5)	0.712
<65 years	38	16(42.1)	22(57.9)	
Sex				
Male	159	72(45.3)	87(54.7)	1.000
Female	6	3(50)	3(50)	
LN metastasis				
Negative	66	32(48.5)	34(51.5)	0.529
Positive	99	43(43.4)	56(56.6)	
Tumor size				
≥4.0 cm	94	43(45.7)	51(54.3)	1.00
<4.0 cm	71	32(45.1)	39(54.9)	
TNM Stage				
I	27	14(51.9)	13(48.1)	0.631
II	57	26(45.6)	31(54.4)	
III	60	28(46.7)	32(53.3)	
IV	21	7(33.3)	14(66.7)	
Differentiation				
W/D	27	16(59.3)	11(40.7)	0.039
M/D	108	51(47.2)	57(52.8)	
P/D	30	8(26.7)	22(73.3)	
Chemotherapy				
Absent	54	22(40.7)	32(59.3)	0.411
Positive	111	53(47.7)	58(52.3)	
Radiation Therapy				
Absent	94	49(52.1)	45(47.9)	0.058
Positive	71	26(36.6)	45(63.4)	

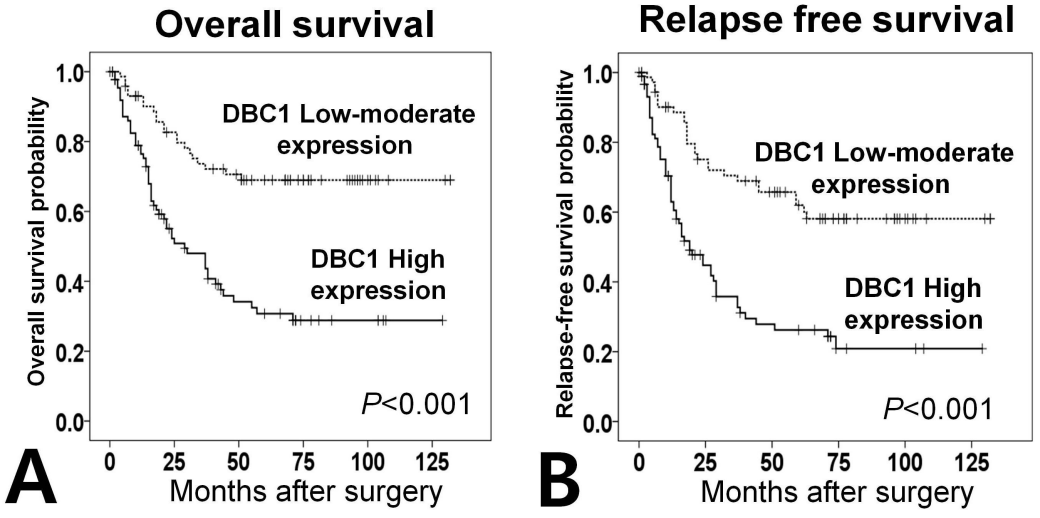

Fig. 2. Kaplan-Meier curves illustrating overall survival **(A)** and relapse-free survival **(B)** among ESCC patients on the basis of DBC1 expression status. Both overall and disease-free survivals were significantly worse in patients with a high expression of DBC1 (P<0.001).

significantly associated with shorter overall survival and relapse-free survival by univariate analysis (p<0.001 for both, Table 3).

The results of a Kaplan-Meier univariate analysis are shown in Figure 2. The overall and relapse-free survival curves with respect to DBC1 expression are shown. Both

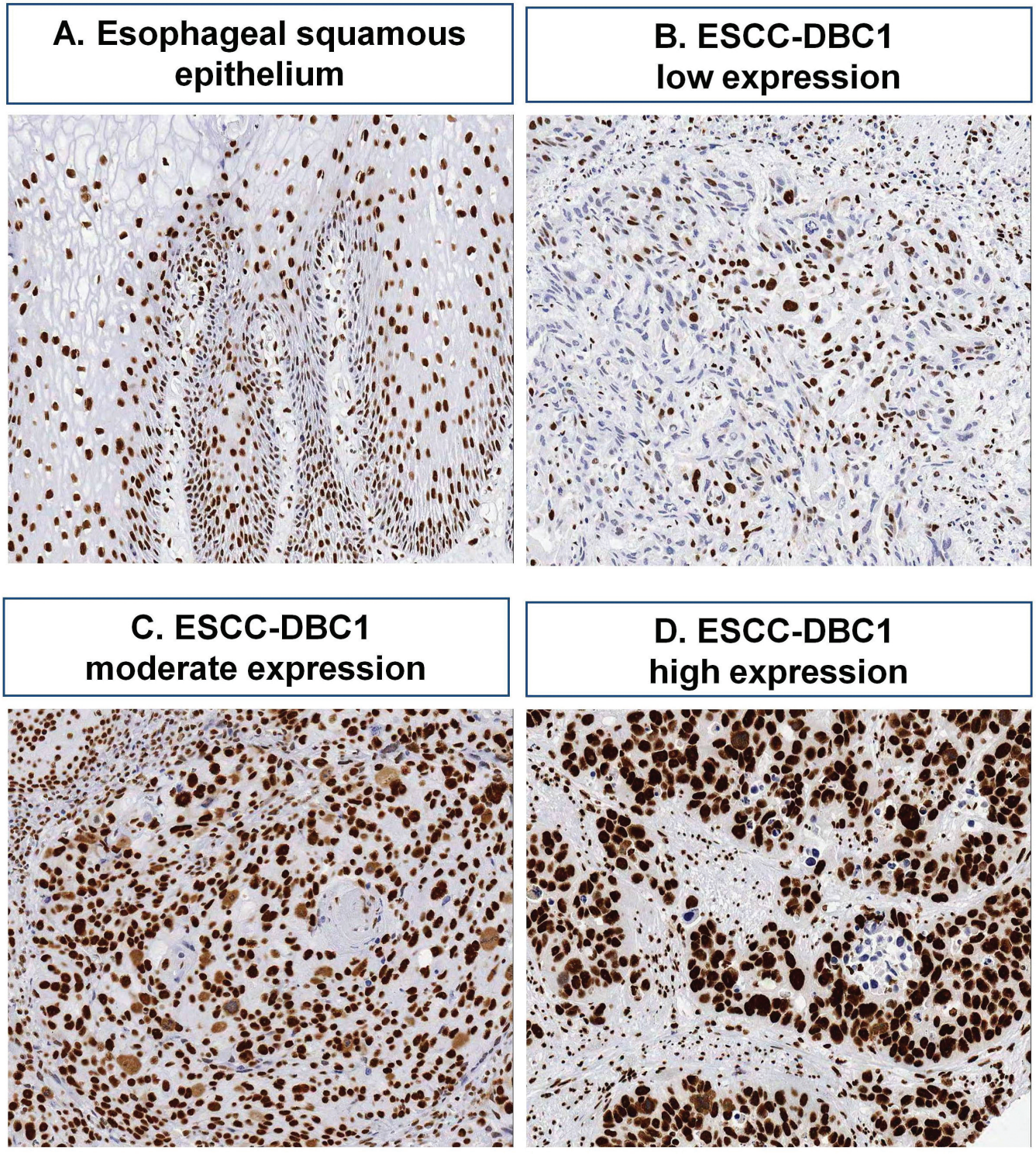

Fig. 1. Representative immunohistochemical staining in normal esophagus and ESCC for DBC1.

Table 3. Univariate Cox regression analysis of relapse-free survival and overall survival.

	Case no.	OS		RFS	
		HR (95% CI)	P	HR (95% CI)	P
Age					
<65 years	38	1		1	
≥65 years	127	1.120 (0.655-1.916)	0.679	1.030 (0.631-1.681)	0.906
LN metastasis					
Negative	66	1		1	
Positive	99	2.519 (1.516-4.186)	0.000	2.586 (1.596-4.190)	<0.001
Tumor size					
≥4.0 cm	94	1		1	
<4.0 cm	71	1.565 (0.990-2.474)	0.055	1.540 (1.001-2.370)	0.049
TNM Stage					
I	27	1		1	
II	57	3.768 (1.128-12.591)	0.031	2.579 (0.987-6.738)	0.053
III	60	8.084 (2.499-26.150)	<0.001	5.054 (1.996-12.801)	0.001
IV	21	9.743 (2.797-33.938)	<0.001	7.725 (2.863-20.846)	<0.001
Differentiation					
W/D	27	1		1	
M/D	108	1.930 (0.918-4.061)	0.083	2.009 (0.999-4.040)	0.050
P/D	30	2.847 (1.237-6.551)	0.014	2.447 (1.089-5.496)	0.030
DBC1					
Low to moderate	75	1		1	
High	90	3.142 (1.890-5.222)	<0.001	2.889 (1.822-4.582)	<0.001
Chemical Therapy					
Absent	54	1		1	
Positive	111	0.862 (0.545-1.362)	0.525	0.988 (0.636-1.535)	0.957
Radiation Therapy					
Absent	97	1		1	
Positive	71	2.195 (1.404-3.432)	0.001	2.350 (1.542-3.582)	0.001

OS, overall survival RFS, Relapse free survival.

Table 4. Multivariate Cox regression analysis of relapse-free survival and overall survival.

Predictors	OS		RFS	
	HR (95% CI)	P	HR (95% CI)	P
Age				
0-65	1.00		1.00	
65+	1.191 (0.653~2.172)	0.569	1.184 (0.686~2.041)	0.544
TNM Stage				
I	1.00		1.00	
II	3.217 (0.957~10.809)	0.059	1.980 (0.750~5.233)	0.168
III	6.828 (2.095~22.254)	0.001	3.786 (1.475~9.720)	0.006
IV	9.744 (2.765~34.340)	<0.001	6.389 (2.342~17.434)	<0.001
Chemotherapy				
absent	1.00		1.00	
positive	0.873 (0.521~1.464)	0.606	1.029 (0.627~1.687)	0.911
Radiation therapy				
absent	1.00		1.00	
positive	1.975 (1.232~3.167)	0.005	2.088 (1.342~3.249)	0.001
DBC1				
Low to moderate	1.00		1.00	
High	2.830 (1.680~4.767)	<0.001	2.655 (1.650~4.274)	<0.001

OS, overall survival RFS, Relapse free survival.

overall survival (Fig. 2A) and relapse-free survival (Fig. 2B) in patients with DBC1 overexpression were significantly lower than in patients with low to moderate DBC1 expression (p<0.001 for both).

Multivariate analysis of prognostic variables in ESCC patients

Multivariate analyses of overall and relapse-free survival rates of esophageal cancer patients were performed using Cox proportional-hazards regression. In the multivariate overall survival analyses, age, TNM stage, history of chemotherapy and radiation therapy, and DBC1 expression were entered into a Cox proportional-hazard analysis. High expression of DBC1 (relative risk: 2.830; p<0.001), TNM stage and history of radiation therapy were identified as independent predictive factors (Table 4). In the multivariate relapse-free survival analysis, including the same set of variables, high expression of DBC1 was also identified as an independent predictive factor (Table 4).

DBC1 expression in ESCC cell lines

DBC1 expression in three ESCC cell lines (TE1, TE8, and TE10) was determined using both western blotting and real-time RT-PCR. Western blotting revealed that all of these cell lines strongly express DBC1 protein. In all three cell lines single and strong protein bands were detected at approximately the 120kDa (Fig. 3B). Real-time RT-PCR showed that DBC1 RNA was also highly upregulated in all three ESCC cell lines, and this result is consistent with that of western blotting. Specifically, the amplified DNA products from the mRNA of these cell lines were detected using cybergreen and were quantified by real-time PCR (Fig. 3A). Ct values of GADPH (reference gene) ranged from 13.7 to 14.5, and Ct values of DBC1 ranged from 16.7 to 18.8. The DBC1 mRNA level was normalized against the reference gene (GAPDH), and the ratio of DBC1 concentration to the reference gene (GAPDH) ranged from 0.056 to 0.12.

Knockdown of DBC1 reduced proliferative, migratory, and invasive abilities of ESCC cell line

To identify the functional role of DBC1 in tumor cells, we knocked down DBC1 in TE10 using siRNA and assessed its effect on the proliferative, migratory, and invasive abilities of tumor cells. As a first step, we designed and tested siRNA against DBC1. As shown in Figure 4A, DBC1 expression was greatly reduced during siRNA treatment at a concentration of 10 nM. Then, we performed MTT assay to examine proliferative ability of cancer cells (Fig. 4B) and in vitro colony formation assay to evaluate tumorigenic ability (Fig. 4C). As a result, the proliferative activity of cancer cells was significantly decreased in DBC1-knocked down TE10 cells. In addition, tumorigenic ability was also significantly reduced in DBC1-knocked down TE10 cells. Invasion and migration assays were then performed with these DBC1-expressing / DBC1-deleted TE10 cells. As shown in Figure 4D, both migratory and invasive ability were dramatically reduced in cells when DBC1 was down-regulated by siRNA treatment. These results suggest that DBC1 may enhance both the migration and invasion of ESCC.

Discussion

In this study, we examined the expression of DBC1 in normal and ESCC tissues and assessed its prognostic value. We demonstrated that DBC1 expression was significantly associated with poor survival in both univariate and multivariate analyses (p<0.001) and this is the first report to study the clinical significance of DBC1 as a prognostic factor in ESCC. This result is highly consistent with recent studies that DBC1 is significantly correlated with a poor prognosis in gastric (Cha et al., 2009) and breast carcinoma (Sung et al.,

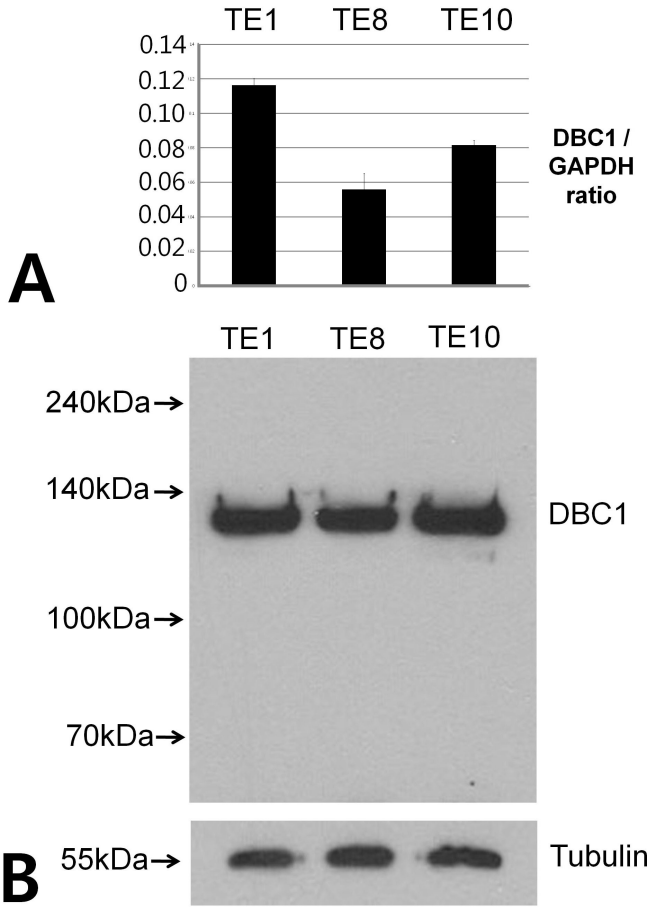

Fig. 3. DBC1 mRNA and protein expression in ESCC cell lines. **A.** Amplification curve for the PCR product by real time RT-PCR. **B.** Result of western blotting analysis.

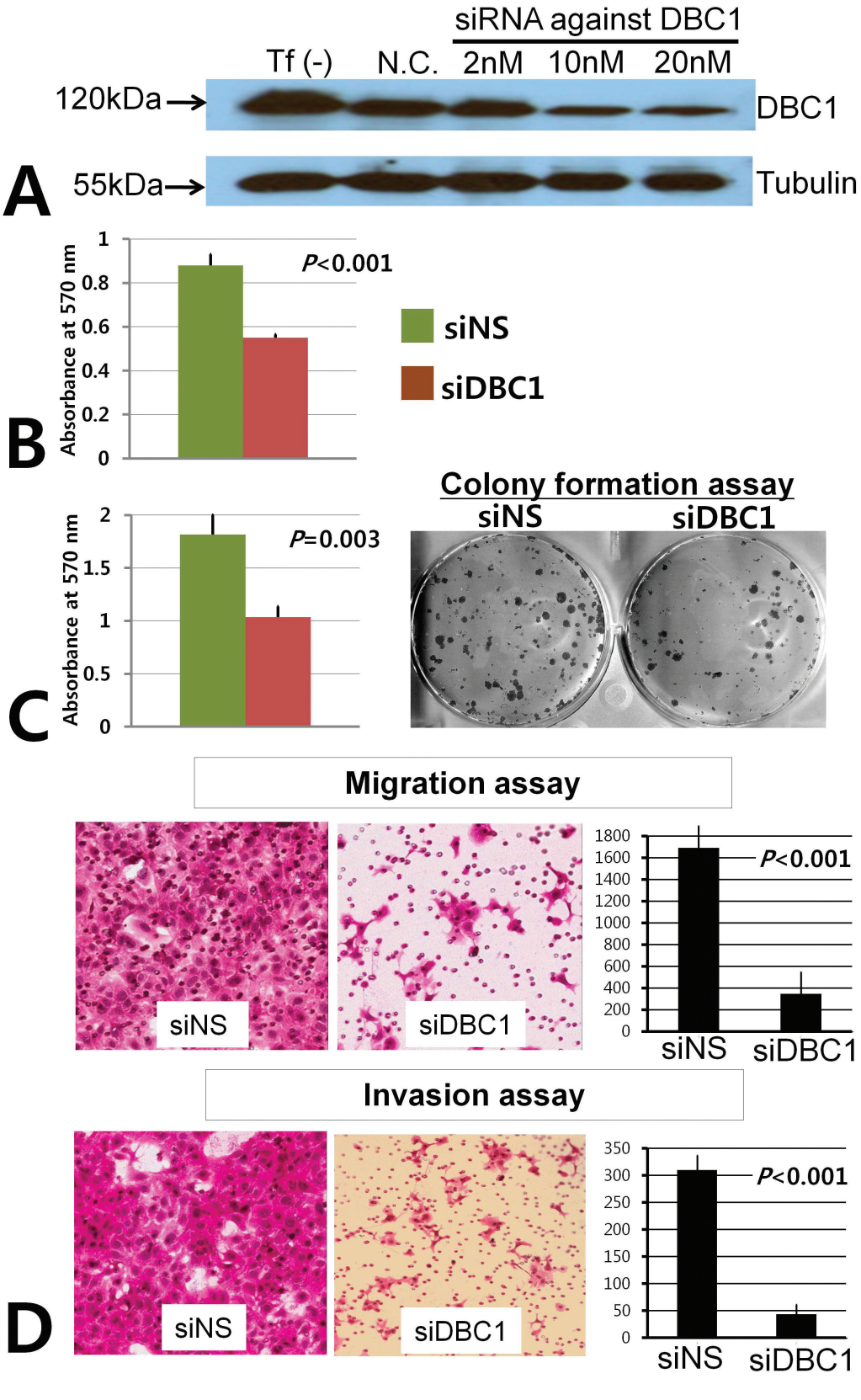

Fig. 4. The effect of DBC1 depletion on proliferation, migration, and invasion of ESCC cell line (TE10). **A.** Depletion of DBC in TE10 by transfection of siRNA against DBC1. **B.** MTT assay revealed that the proliferative activity of cancer cells was significantly decreased in TE10 cells with transfection of siDBC1 (siRNA against DBC1) than in TE10 cells with Transfection of siNS (Non Specific siRNA). **C.** Colony formation assay showed that tumorigenic ability was also significantly reduced in cancer cells when siDBC1 was transfected than when siNS was transfected. **D.** Migration and invasion of TE10 was greatly reduced after siRNA transfection. Scrambled siRNA was used as a negative control.

2010; Lee et al., 2011).

Although silencing of DBC1 has been reported in several cancers, including non-small cell lung cancer (Izumi et al., 2005), we observed the prevalent expression of DBC1 in both ESCC tissues and ESCC cell lines. Therefore, the silencing of DBC1 is estimated to be rare in ESCC. In stomach, the DBC1 expression rate was reported to be 2/10 (20%) in normal gastric epithelium and 45% in gastric cancer (Cha et al., 2009). However, in our study, all normal and cancerous esophageal epithelium expressed DBC1, despite the variability in the extent and intensity of staining. DBC1 was also strongly expressed in all three esophageal cancer cell lines. Therefore, DBC1 expression seems to be more prevalent in esophageal epithelium than in gastric epithelium.

In our study, most of the DBC1 staining was observed in the nucleus, and cytoplasmic staining of DBC1 was infrequently observed. However, its cytoplasmic staining pattern was unique, usually being observed in abnormal cells with shrunken nucleus and large cytoplasm. According to Sundararajan et al. (Sundararajan et al., 2005) DBC1 is localized in the nucleus in healthy cells but is localized in the cytoplasm during apoptosis. In the same study, endogenous DBC1 was reported to undergo caspase dependent cleavage during TNFα mediated death signaling. As a result, truncated DBC1 was generated and it sensitized TNFα induced apoptosis. Considering these findings, these cytoplasmic DBC1 could have a truncated form and may be involved in apoptosis.

The mechanism by which DBC1 affects survival of ESCC patients is largely unknown. The results of our *in-vitro* study using ESCC cell lines indicates that DBC1 may enhance the aggressive behavior of cancer cells in ESCC patients. In our *in-vitro* study, DBC1 was highly expressed in ESCC cell lines and the depletion of DBC1 by siRNA leads to a reduction in the migration and invasion of tumor cells. These results suggest that DBC1 may enhance both the migration and invasion of ESCC. However, the mechanism underlying this decreased migration and invasion of cancer is still unknown.

The exact role of DBC1 in tumorigenesis has been poorly understood and is still controversial. Kim et al. and Zhao et al. reported that DBC1 is a negative regulator of SIRT1 (Kim et al., 2008; Zhao et al., 2008). SIRT1 was initially known as a tumor-promoter and, consequently, DBC1 was assumed to be a tumor suppressor. However, it was recently reported in a series of studies that SIRT1 sometimes functions as a tumor suppressor instead of a tumor promoter under certain conditions (Firestein et al., 2008; Wang et al., 2008; Kabra et al., 2009; Yuan et al., 2009). Considering these data, SIRT1 may function as both a tumor promoter and a tumor suppresser (Kim et al., 2009). Given that DBC1 is a major negative regulator of SIRT1, it is entirely possible that DBC1 may also participate in both tumor promotion and tumor suppression (Kim et al., 2009). In fact, it has been suggested that DBC1 promotes tumor apoptosis after caspase-dependent cleavage (Sundararajan et al., 2005). However, in Trauernicht et al.'s study the depletion of DBC1 by siRNA enhances the death of MCF-7 breast cancer cells, which suggests a positive role of DBC1 in cell proliferation (Trauernicht et al., 2007).

These contradictory results are speculated to originate from the function of DBC1 itself. Specifically, DBC1 is basically a cotranscription factor that binds to and modulates other transcription factors, and DBC1 itself does not have a specific function. Therefore, the nature of the main transcription factor complex to which DBC1 binds will decide its overall function. In addition to SIRT1, DBC1 is known to modulate various transcription factors, such as retinoic acid receptor α (RARα), estrogen receptor and androgen receptor etc (Kim et al., 2009). To determine the fundamental mechanism of DBC1 in ESCC, the major transcription complex to which DBC1 binds must be studied.

In conclusion, DBC1 is a strong independent prognostic predictor of clinical outcome in esophageal cancer and enhances the migration and invasion of ESCC cells.

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