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Increased expression of Na^+/H^+ exchanger isoform 1 predicts tumor aggressiveness and unfavorable prognosis in epithelial ovarian cancer

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Abstract. Na^+/H^+ exchanger isoform 1 (NHE1), which is a regulator of intracellular and extracellular pH via ion exchange, has been demonstrated to serve an important role in cell differentiation, migration and invasion in solid tumors and hematological malignancies. However, the potential role of NHE1 in epithelial ovarian cancer (EOC) remains unclear. In the present study, the expression pattern and the prognostic value of NHE1 were investigated in EOC. EOC tissues, non-cancerous tumors and normal ovarian tissues were collected, and the expression levels of NHE1 were determined using the reverse transcription-quantitative polymerase chain reaction, western blotting and immunohistochemistry. The expression pattern of NHE1 was also evaluated in ovarian cancer cell lines using western blotting and immunofluorescence. In addition, the association between the NHE1 expression pattern and the clinicopathological features and the clinical prognosis of patients with EOC was also analyzed. The expression levels of NHE1 were identified to be significantly increased in EOC tissues compared with non-cancerous tumors and normal ovarian tissues ($P<0.05$). Furthermore, the increased expression of NHE1 was associated with an advanced International Federation of Gynecology and Obstetrics stage (FIGO III-IV; $P<0.001$) and the presence of high-grade carcinoma (grades 2-3, $P<0.001$). Overexpressed NHE1 was identified as a risk factor of shorter PFS ($P<0.001$) and OS ($P<0.001$). A multivariate Cox's regression analysis revealed that NHE1 was an independent prognostic factor for the prediction of the outcome of patients with EOC. NHE1 may, therefore, serve as a potential therapeutic target to inhibit tumor aggressiveness.

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Abbreviations: EOC, epithelial ovarian cancer; NHE1, Na^+/H^+ exchanger isoform 1; FIGO, the International Federation of Gynecology and Obstetrics; PFS, progression-free survival; OS, overall survival

Key words: Na^+/H^+ exchanger isoform 1, epithelial ovarian cancer, prognosis, invasion, survival rate

Introduction

Ovarian cancer is the leading cause of cancer-associated mortality resulting from gynecological tumors in the USA. The American Cancer Society estimated that 22,280 females would develop ovarian cancer in 2016 and that 14,240 females may succumb to the disease (1). Epithelial ovarian cancer (EOC) accounts for >80% of all cases of ovarian cancer. Among patients with EOC, ~3/4 cases are diagnosed in patients with stage III or IV disease due to the lack of sensitive detection methods or prominent symptoms; in addition, patients in these later disease stages exhibit a 5-year survival rate of <30%. Molecular targeted therapeutic drugs, including bevacizumab and olaparib, have been confirmed to improve progression-free survival (PFS) rates in women with EOC, but they do not increase overall survival (OS) rates (2-8). Furthermore, the cost of these drugs means that patients in developing countries may not be able to afford them. Therefore, complete resection with no residual disease is a critical factor for the improvement of the prognosis of patients with advanced EOC. However, it is difficult to completely resect the lesions of advanced EOC on account of widespread intra-abdominal metastases and peritoneal implantation. Consequently, molecular changes associated with the metastasis of EOC may be identified to provide novel targets for intervention.

A common feature of tumors is the dysregulation of pH control (9). To sustain tumor growth, cancer cells need to adapt to the tumor-associated acidic microenvironment. Under these circumstances, the activation of Na^+/H^+ exchanger isoform 1 (NHE1) is crucial for the control of intracellular pH (pHi). NHE1 is a ubiquitous membrane protein that is known to regulate pH homeostasis via the electroneutral exchange of one intracellular H^+ ion for one extracellular Na^+ ion (10,11). Intracellular alkalinization and acidification of the

microenvironment caused by NHE1 serve an important role in cell migration, invasion, proliferation, differentiation and apoptosis in solid tumors and hematological malignancies, including breast cancer (12,13), hepatocellular carcinoma (14,15), pancreatic ductal adenocarcinoma (16), cervical cancer (17) and acute myeloid leukemia (18). Regarding triple-negative breast cancer, NHE1 inhibition increases the efficacy of paclitaxel in MDA-MB-231 cells and decreases their viability as well as their migratory and invasive potential *in vitro*. Furthermore, the knockout of NHE1 markedly decreases *in vivo* xenograft tumor growth of MDA-MB-231 cells in athymic nude mice (12).

A previous study, which used reverse capture antibody microarray technology to identify plasma autoantibodies from patients with mucinous ovarian cancer, revealed significant overexpression of NHE1 in plasma samples obtained from patients with cancer compared with those obtained from healthy controls (19). However, little research has been performed on the role of NHE1 in the development and progression of EOC. In the present study, the expression pattern of NHE1 was detected in human EOC tissues and human ovarian cancer cell lines. In addition, the prognostic value of NHE1 in EOC was analyzed.

Materials and methods

Patients and samples. A total of 184 formalin-fixed paraffin-embedded tissue samples consisting of 129 EOCs, 18 borderline tumors, 22 benign tumors and 15 normal ovarian tissues, were retrieved from the archives of the Department of Pathology, Chongqing Medical University (Chongqing, China), from February 2005 to December 2010. Fresh surgical specimens, which were obtained from 52 patients with epithelial ovarian tumors and 10 patients with normal ovaries, were snap-frozen in liquid nitrogen immediately following surgery performed between October 2011 and December 2012 and stored at -80˚C. The epithelial ovarian tumor samples comprised 28 EOCs, 10 borderline tumors and 14 benign tumors. Normal ovarian tissue samples were collected from patients who underwent hysterectomy for non-ovarian diseases. All patients had undergone cytoreductive surgery as a primary treatment.

The specific clinicopathological features of patients with EOC who provided samples for immunohistochemical staining are summarized in Table I. Surgical staging was based on the International Federation of Gynecology and Obstetrics (FIGO) staging system. The carcinoma grade was subdivided into low (G1) and high (G2/G3) grade. PFS and OS rates were calculated from the date of initial diagnosis to the date of progression/mortality or the date of the last follow-up. Ethical approval for the present study was obtained from the local ethics committee. Prior written informed consent was obtained from the patients who participated in the present study in accordance with The Declaration of Helsinki.

Immunohistochemistry. Tumor tissues were fixed in 10% neutral-buffered formalin for 24 h at room temperature, embedded in paraffin. Immunohistochemical analysis of NHE1 was conducted on 4-μm-thick formalin-fixed paraffin-embedded specimens. The slides were deparaffinized in xylene and rehydrated in graded solutions of alcohol. Antigen retrieval was performed by treating the sections with citric acid (pH 6.0) for 20 min. Non-specific proteins were blocked by incubating the slides with 5% bovine serum albumin (Beyotime Institute of Biotechnology, Haimen, China) for 30 min at room temperature. The sections were then incubated overnight at 4˚C with a primary rabbit polyclonal antibody against human NHE1 (1:100 dilution; cat. no. ab67314; Abcam, Cambridge, MA, USA). Next, the slides were incubated with the appropriate biotinylated secondary antibody for 30 min at 37˚C. Following washing, the slides were incubated with strepavidin-biotin complex reagent (SA1022, Boster Biological Technology, Pleasanton, CA, USA) followed by development with 3,3'-diaminobenzidine solution.

All tissue sections were randomly evaluated by two independent blinded pathologists, Dr Rui Chen and Dr Jue Xiao, from the Departments of Pathology, Chongqing University Cancer Hospital and Institute and Cancer Center (Chongqing, China) . NHE1 expression in EOC was evaluated using an inverted microscope by scanning the entire tissue specimen under low magnification (magnification, x40), and was confirmed under high magnification (magnification, x200). NHE1 staining was predominantly localized within the membrane and cytoplasm. Immunostaining for NHE1 was scored using a semiquantitative scale through the evaluation of the staining intensity (0, absent; 1, weak; 2, moderate; 3, strong) and the proportion of positive tumor cells (0, absent; 1, <33%; 2, 33-66%; 3, >66%). The staining intensity score was multiplied by the percentage score to obtain the total score (0, 1, 2, 3, 4, 6 and 9). Scores between 0 and 4 were defined as low NHE1 expression, whereas scores between 6 and 9 were defined as high NHE1 expression.

Cell culture. Distinct tumor-derived human ovarian cancer cell lines (OVCAR-3, 3AO, SKOV3 and A2780) were used in the present study. OVCAR-3 (serous) and 3AO (mucinous) were purchased from the Chinese Academy of Sciences Type Culture Collection (Shanghai, China). SKOV3 (papillary serous) and A2780 (adenocarcinoma) were kindly provided by Dr Hua Linghu (Department of Obstetrics and Gynecology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China) and West China Second Hospital (Sichuan University, Chengdu, China), respectively. All cell lines were cultured as monolayers in RPMI-1640 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (GE Healthcare Life Sciences, Logan, UT, USA) and 1% penicillin/streptomycin (Beyotime Institute of Biotechnology) at 37˚C in a humidified incubator containing 5% CO_2.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated with TRIzol reagent (TaKaRa Bio, Inc., Otsu, Japan), according to the manufacturer's protocol. In total, 1 μg RNA was reverse-transcribed into cDNA using the PrimeScript II First Strand cDNA Synthesis kit (Takara Bio, Inc.), according to the manufacturer's protocols. qPCR was performed in a CFX96™ Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with a SYBR® PrimeScript® RT-PCR kit

Table I. Clinicopathological features in 129 patients with EOC according to NHE1 expression.

Clinicopathological parameters	n	NHE1 Expression		P-value
	n=129	Low (%)	High (%)	
Age, years				0.445
<50	61	11 (18.0)	50 (82.0)	
>50	68	16 (23.5)	52 (76.5)	
Serum CA-125 , U/ml				0.792
<35	17	4 (23.5)	13 (76.5)	
>35	112	23 (20.5)	89 (79.5)	
FIGO stage				<0.001[a]
I/II	37	17 (45.9)	20 (54.1)	
III/IV	92	10 (10.9)	82 (89.1)	
Grade				<0.001[a]
G1	23	11 (47.8)	12 (52.2)	
G2/G3	106	16 (15.1)	90 (84.9)	
Histological type				
Serous	74	13 (17.6)	61 (82.4)	
Mucinous	17	5 (29.4)	12 (70.6)	
Clear cell	9	2 (22.2)	7 (77.8)	
Endometrioid	29	7 (24.1)	22 (75.9)	
Serous vs. non-serous				0.278
Ascites, ml				0.129
<100	46	13 (28.3)	33 (71.7)	
>100	83	14 (16.9)	69 (83.1)	
Residual disease, cm				0.438
<1	103	23 (22.3)	80 (77.7)	
>1	26	4 (15.4)	22 (84.6)	

[a]P<0.05. FIGO, the International Federation of Gynecology and Obstetrics; NHE1, Na^+/H^+ exchanger isoform 1.

(TaKaRa Bio, Inc.). The PCR thermocycling conditions were 95˚C for 30 sec followed by 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec.

The following primers were used: Human NHE1 forward 5'-GCCTTCTCTCTGGGCTACCT-3' and reverse 5'-CTTGTCCTTCCAGTGGTGGT-3'; human GAPDH forward 5'-AATGTCCCAGAGTGTGCCGAG-3' and reverse 5'-ATGCCTTGCCGACCGTGTA-3'. GAPDH was used as a reference gene. qPCR results were quantified according to the $2^{-\Delta\Delta Cq}$ method (20).

Western blotting. Western blotting was performed as previously described (21). Briefly, frozen tissue samples and cell lines were homogenized on ice in radioimmunoprecipitation assay lysis buffer (cat. no., P0013B, Beyotime Institute of Biotechnology) consisting of 50 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and protease inhibitor mixture, including sodium orthovanadate, sodium fluoride,EDTA and leupeptin. The samples were then centrifuged at 12,000 x g at 4˚C for 10 min to remove cellular debris. Following quantification of the protein extracts using bicinchoninic acid protein assay, equivalent amounts of protein (50 μg/lane) was loaded onto 10% acrylamide gels for SDS-PAGE and then electrotransferred onto a polyvinylidene difluoride membrane (Merck KGaA, Darmstadt, Germany). The membrane was blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 for 1 h at 37˚C, which was followed by incubation with a primary rabbit polyclonal antibody against human NHE1 (dilution, 1:1,000; cat. no., ab67314; Abcam) and a mouse monoclonal antibody against human GAPDH (dilution, 1:1,000; cat. no., AG019; Beyotime Institute of Biotechnology) at 4˚C overnight. Subsequent to washing, the membranes were incubated with secondary antibodies, horseradish peroxidase (HRP)-labeled Goat Anti-Rabbit IgG (dilution, 1:1,000; cat. no. A0208; Beyotime Institute of Biotechnology) and HRP-labeled Goat Anti-Mouse (dilution, 1:1,000; cat. no., A0216; Beyotime Institute of Biotechnology) for 1 h at 37˚C. The immunoreactivity was detected using enhanced chemiluminescence plus detection reagents (P0018; Beyotime Institute of Biotechnology). GAPDH served as the loading control.

Immunofluorescence. Cells were plated on sterilized coverslips in a 24-well plate ($2x10^4$ cells/ml) and allowed to adhere for 24 h. The cells were fixed in 4% paraformaldehyde at room temperature for 20 min, followed by permeabilization with

1% Triton-X-100 at 37˚C for 10 min. The cells were blocked with 5% normal goat serum at 37˚C for 30 min and were then incubated with the rabbit anti-NHE1 antibody (1:100) at 4˚C overnight. Subsequently, the cells were incubated with anti-rabbit FITC conjugated secondary antibody (dilution, 1:500; cat. no., ab6717; Abcam, Cambridge, MA, USA) at 37˚C for 1 h, and propidium iodide was used to label the nuclei. Finally, fluorescence images were captured using a laser-scanning confocal microscope.

Statistical analysis. SPSS software was used for statistical analysis (version 17.0; SPSS, Inc., Chicago, IL, USA). For continuous variables, the data are presented as the mean ± standard deviation and an independent Student's t-test was performed. The association between NHE1 immunohistochemical staining and the clinicopathological features was analyzed using the Mann-Whitney U test. Survival rates were analyzed using the Kaplan-Meier estimator method, and the difference in survival rates between patients whose tumors demonstrated high and low NHE1 expression was determined using a log-rank test. A multivariate analysis of survival rates was performed using the Cox's hazard model. All tests were two-tailed, and P<0.05 was considered to indicate a statistically significant difference.

Results

NHE1 is overexpressed in patients with EOC. To investigate the effect of NHE1 on the determination of the clinical prognosis of patients with EOC, the expression of NHE1 in 184 paraffin-embedded tissue samples was detected. Immunohistochemical assays revealed that NHE1 was primarily localized within the cytomembrane and the cytoplasm of EOC tissue samples. High expression of NHE1 was revealed in 102/129 (79.7%) EOC tissues compared with only 5/18 (27.8%) borderline tumor tissues (P<0.01), 7/22 (31.8%) benign tumor tissues (P<0.01) and 4/15 (26.7%) normal ovarian tissues (P<0.01; Fig. 1A). Furthermore, immunohistochemical assays also revealed high expression of NHE1 in samples that represented four different histological types including serous, mucinous, clear cell and endometrioid tumors (Fig. 1B). No difference was observed among histological types with respect to NHE1 protein expression (P>0.05; Table I).

The increased expression of NHE1 was also confirmed in an additional 28 frozen EOC tissues using RT-qPCR and western blotting. The relative expression of NHE1 mRNA in EOC tissues (5.348±1.054) was increased compared with that in the borderline tumor tissues (2.242±0.683; P<0.01), benign tumor tissues (1.936±0.932; P<0.01) and normal ovarian tissues (1.794±0.539; P<0.01; Fig. 2A). The levels of NHE1 protein were also significantly increased in EOC tissues (0.841±0.208) compared with borderline tumors (0.598±0.182; P=0.002), benign tumors (0.534±0.164; P<0.01) and normal ovarian tissues (0.494±0.149; P<0.01; Fig. 2B).

Increased expression of NHE1 is associated with tumor progression. To further explore the clinicopathological features of NHE1-positive tumors, the relevance between the level of NHE1 protein and specific clinicopathological features [including age at diagnosis, serum cancer antigen (CA)-125 level, FIGO stage, grade, histological type, ascites and residual disease] in 129 EOC samples was evaluated. As presented in Table I, NHE1 immunoreactivity was significantly increased in samples with FIGO stage III/IV (FIGO stage III/IV vs. I/II; P<0.001) and high-grade carcinoma (grade 2-3 vs. grade 1; P<0.001). No association between NHE1 protein expression and age at diagnosis, serum CA-125 level, histological type, presence of ascites or residual disease was identified.

NHE1 is expressed in the major subtypes of human EOC-derived cell lines. Considering that the immunohistochemistry results demonstrated increased expression of NHE1 in human EOC tissues, the level of NHE1 protein was analyzed in various EOC cell lines using western blotting. The results revealed that NHE1 was highly expressed at various levels in SKOV3, OVCAR-3, A2780 and 3AO cells (Fig. 3A). Next, the localization of NHE1 was also examined in OVCAR-3 cells using immunofluorescence, and the results revealed that NHE1 was localized predominantly within the cytomembrane and the cytoplasm (Fig. 3B).

Increased expression of NHE1 predicts poor prognosis in EOC. The association of NHE1 overexpression with the clinical prognosis of 129 patients with EOC was analyzed. Following a 5-year follow-up, the survival rates of patients whose tumors expressed low and high levels of NHE1 were 74.1 and 36.3%, respectively. To assess whether NHE1 may serve as a predictor of survival rate in EOC, a Kaplan-Meier estimator analysis was performed to explore the association between the level of NHE1 expression and patient survival rate. The log-rank test revealed that patients with high expression of NHE1 had a shorter PFS/OS than those with low expression of NHE1 (Fig. 4A and B). According to a univariate analysis, high expression of NHE1 was associated with a shorter OS [hazard ratio (HR), 4.212; 95% confidence interval, 1.922-9.230; P<0.001] in EOC (Table II). According to a multivariate survival rate analysis, NHE1 remained a significant factor when age, serum CA-125 level, FIGO stage, grade, histological type, presence of ascites and residual disease were used as covariates (HR, 0.402; 95% confidence interval, 0.173-0.993; P=0.034; Table II).

Discussion

The ability to alter the pH is a characteristic of tumor cells. *In vivo* and *in vitro* experiments have demonstrated that tumor cells exhibit an alkaline pHi (7.12-7.65 in tumor cells vs. 6.99-7.20 in normal tissues) and an acidic extracellular pH (pHe; 6.2-6.9 vs. 7.3-7.4) (9). The acidic tumor microenvironment is hypothesized to accelerate extracellular matrix remodeling, which results in metastasis (22). NHE1, as an isoform of the Na^+/H^+ exchanger family (comprising NHE1-NHE9), has been detected in the plasma membrane of epithelial cells and has been demonstrated to be a crucial regulator of pHi and pHe via ion exchange (23).

Previous studies indicate that the overexpression of the NHE1 protein and the dysregulation of NHE1 activity are associated with tumor malignancy (24,25). To the best of our knowledge, the association between NHE1 and metastasis has not previously been investigated in EOC. The present study

Table II. Univariate and multivariate analyses of the factors that affect the overall survival rate of patients with endothelial ovarian cancer.

Clinicopathological parameters	n	Univariate analysis		Multivariate analysis	
	n=129	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years					
<50	61	Reference		Reference	
>0	68	1.023 (0.643-1.627)	0.925	1.435 (0.848-2.428)	0.178
Serum CA-125, U/ml					
<35	17	Reference		Reference	
>35	112	0.794 (3.739-23.249)	0.483	1.159 (0.499-2.693)	0.731
FIGO stage					
I/II	37	Reference		Reference	
III/IV	92	9.324 (3.739-23.249)	<0.001[a]	0.117 (0.045-0.036)	<0.001[a]
Grade					
G1	23	Reference		Reference	
G2/G3	106	14.339 (3.495-58.824)	<0.001[a]	0.082 (0.019-0.351)	0.001[a]
Histological type					
Serous	74	Reference		Reference	
Serous vs. non-serous	55	1.033 (0.645-1.656)	0.892	0.591 (0.321-1.087)	0.091
Ascites, ml					
<100	46	Reference		Reference	
>100	83	1.058 (0.651-1.721)	0.819	1.875 (0.965-3.645)	0.064
Residual tumor, cm					
<1	103	Reference		Reference	
>1	26	1.889 (1.100-3.276)	0.021[a]	0.594 (0.332-1.063)	0.079
NHE1 expression					
Low	27	Reference		Reference	
High	102	4.212 (1.922-9.230)	<0.001[a]	0.402 (0.173-0.993)	0.034[a]

[a]P<0.05. HR, hazard ratio; CI, confidence interval; FIGO, the International Federation of Gynecology and Obstetrics.

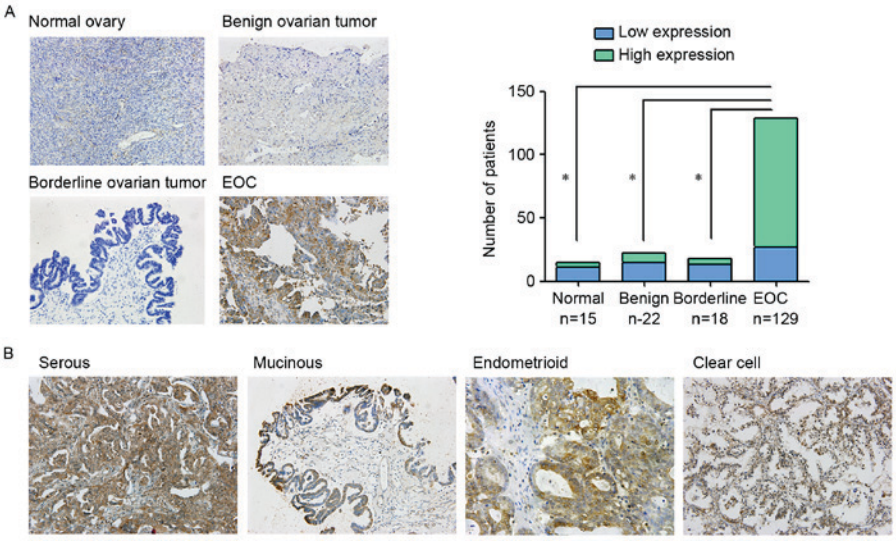

Figure 1. Immunohistochemical staining for NHE1 protein in EOC, non-cancerous tumors and normal ovarian tissue samples. (A) NHE1 was highly expressed in the cytomembrane and cytoplasm of EOC cells. Strong positive staining for NHE1 was observed in EOC tissue samples (n=129), whereas negative or weak immunoreactivity of NHE1 was observed in the borderline (n=18), benign (n=22) and normal ovarian (n=15) tissue samples (magnification, x200). (B) Positive immunoreactivity of NHE1 in different histological types of EOC (magnification, x200). *P<0.05. EOC, epithelial ovarian carcinoma; NHE1, Na^+/H^+ exchanger isoform 1.

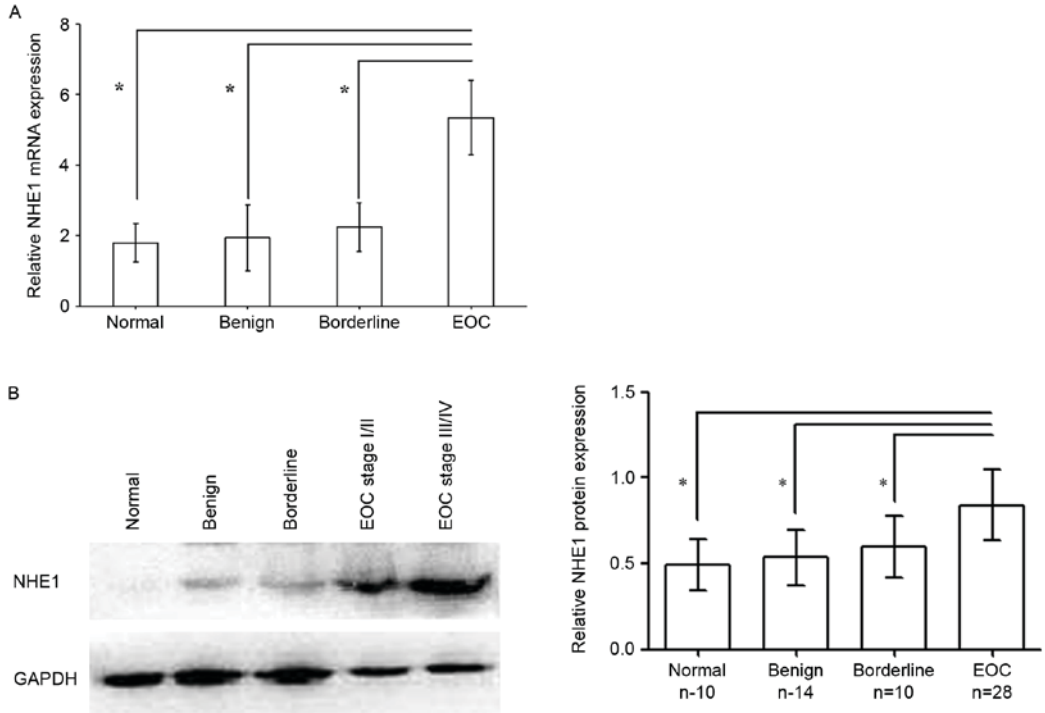

Figure 2. Levels of NHE1 mRNA and protein in freshly frozen EOC tissue (n=28), borderline tumors (n=10), benign tumors (n=14) and normal ovarian tissue (n=15). (A) Reverse transcription-quantitative polymerase chain reaction and (B) western blotting analysis revealed that the levels of NHE1 mRNA and protein in EOC were significantly increased compared with the corresponding levels in non-cancerous tumors and normal ovarian tissues. *P<0.05. EOC, epithelial ovarian carcinoma; NHE1, Na^+/H^+ exchanger isoform 1.

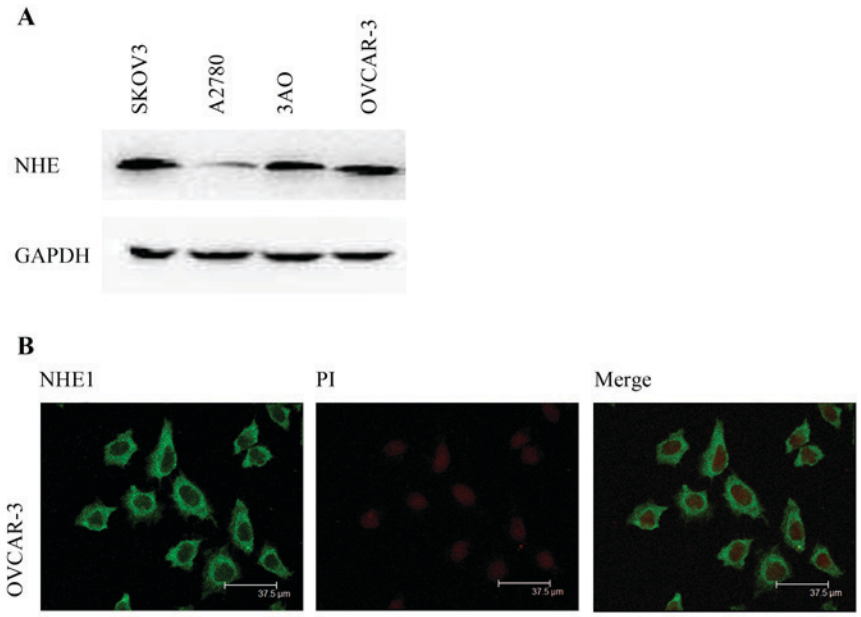

Figure 3. NHE1 is expressed in ovarian cancer cells. (A) Western blotting analysis indicated endogenous NHE1 expression in four cell lines; GAPDH was used as an internal control. (B) Confocal immunofluorescence microscopy was used to capture fluorescence images of NHE1 expression in OVCAR-3 cells: NHE1 expression in OVCAR-3 cells is represented with green fluorescent staining in the cytomembrane and the cytoplasm; the cells were counterstained and the nuclei are identified by red fluorescent staining (magnification, x800). NHE1, Na^+/H^+ exchanger isoform 1; PI, propidium iodide.

explored the expression pattern and prognostic effect of NHE1 in epithelial ovarian tumors of different pathological types and normal ovarian tissues.

The results of the present study revealed that abundant NHE1 protein expression was markedly detected in the cytomembrane and cytoplasm of cancer cells. Furthermore, increased levels of NHE1 mRNA and protein expression were detected in EOC tissues, but not in borderline tumor tissues, benign tumor tissues or normal ovarian tissues. Such results are similar to those of previous studies of NHE1 in breast

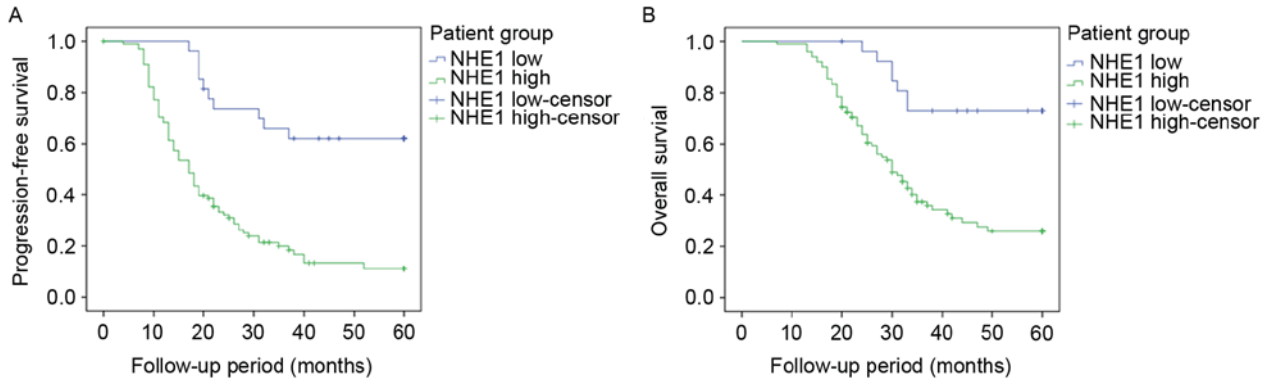

Figure 4. Kaplan-Meier estimator analysis of the association between NHE1 expression and survival rate. The (A) cumulative PFS rate and (B) cumulative OS rate revealed that patients with epithelial ovarian cancer with increased expression of NHE1 protein had a shorter PFS/OS compared with those with low expression. NHE1, Na^+/H^+ exchanger isoform 1; PFS, progression-free survival; OS, overall survival.

cancer (12,13), hepatoma (14,15) and glioblastoma (26,27). The possible association between the NHE1 expression pattern and the specific clinicopathological features of patients with EOC was also analyzed. The results revealed that an increased level of NHE1 expression was associated with advanced FIGO stage and high-grade carcinoma. However, no association was identified between the NHE1 expression pattern and age at diagnosis, serum CA-125 level, histological type, presence of ascites or residual disease. These results suggest that NHE1 may serve an essential role in the development of the transformed phenotype of cancer cells during tumorigenesis. Various studies have investigated the role of NHE1 in the migration and invasiveness of malignant tumors *in vitro*. NHE1 activation in the MDA-MB-435 breast cancer cell line, which is a well-characterized human mammary epithelial cell line that represents late-stage metastatic progression, led to morphological and cytoskeletal changes with increased chemotaxis and cell invasion (28). Furthermore, in MDA-MB-435 breast cancer cells (29) and pancreatic ductal adenocarcinoma cells (16), the inhibition of NHE1 decreased growth and invasive behavior, and during the administration of chemotherapeutic drugs, the antineoplastic effects of those drugs were synergistically strengthened. In summary, it was concluded that the dysregulation of NHE1 may be responsible for the invasive and metastatic behavior of EOC.

Consistent with the results that were obtained from human EOC tissues, the four EOC cell lines examined in the present study exhibited increased levels of NHE1 protein, and according to immunofluorescence assays, the immunoreactivity of NHE1 protein was also localized to the cytomembrane and cytoplasm of ovarian cancer cells. A previous study revealed that NHE1 protein was colocalized with ezrin within lamellipodia and that this protein is possibly associated with the migration of glioma cells (26). It was speculated that the location of NHE1 protein may be connected with the invasiveness and metastasis of EOC cells.

Previous studies have identified a prognostic role for NHE1 protein in malignant tumors (12,13,15,16,26,27,30). The present study investigated the predictive value of NHE1 protein expression in the clinical prognosis of patients with EOC. The results revealed that patients with a high level of NHE1 expression experienced a shorter PFS/OS compared with those with a low level of NHE1 expression. Furthermore, high-grade carcinoma, advanced FIGO stage and suboptimal cytoreductive surgery (residual disease ≥1 cm) were also significantly associated with an increased risk of a poor outcome.

Additionally, a multivariate Cox's regression analysis revealed that NHE1, FIGO stage and carcinoma grade were independent prognostic factors for the prediction of outcomes of patients with EOC. These results suggest that NHE1 may serve as a potential biomarker for the development of EOC. Owing to the relatively small sample size in the present study, further in-depth studies are required to confirm the predictive value of NHE1 protein in EOC.

In summary, the results of the present study revealed that increased expression of NHE1 was identified in EOC tissues and that the overexpression of NHE1 was associated with increased serum CA-125, advanced FIGO stage and high-grade carcinoma. Furthermore, the results indicate that NHE1 may be an independent predictor and risk factor for unfavorable outcome in patients with EOC. These results suggest that a high expression of NHE1 may be an unfavorable prognostic marker of EOC and that NHE1 may serve as a potential therapeutic target for the inhibition of tumor aggressiveness.

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Competing interests

The authors declare that they have no competing interests.

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