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.

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 alkalization 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 University Medical Center (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% CO₂.

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.
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’-GCC TTC TCT CTG GGC TAC CT-3’ and reverse 5’ -CTT GTC CTT CCA GTG GTG GT-3’; human GAPDH forward 5’-AAT GTC CCA GAG TGT GCC GAG-3’ and reverse 5’ -ATG CCT TGC CGA CCG TGT A-3’. GAPDH was used as a reference gene. qPCR results were quantified according to the 2^ΔΔCq 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

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

| Clinicopathological parameters | n=129 | NHE1 Expression | P-value |
|-------------------------------|-------|----------------|---------|
| Age, years                    |       |                |         |
| <50                           | 61    | 11 (18.0) 50 (82.0) | 0.445 |
| >50                           | 68    | 16 (23.5) 52 (76.5)  | 0.792 |
| Serum CA-125, U/ml            |       |                |         |
| <35                           | 17    | 4 (23.5) 13 (76.5)  | <0.001a|
| >35                           | 112   | 23 (20.5) 89 (79.5) |         |
| FIGO stage                    |       |                | <0.001a|
| I/II                          | 37    | 17 (45.9) 20 (54.1) |         |
| III/IV                        | 92    | 10 (10.9) 82 (89.1) |         |
| Grade                         |       |                |         |
| G1                            | 23    | 11 (47.8) 12 (52.2)  | 0.278  |
| G2/G3                         | 106   | 16 (15.1) 90 (84.9) | 0.129  |
| 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         |       |                | 0.048a  |
| Ascites, ml                   |       |                |         |
| <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)  |         |

*P<0.05. FIGO, the International Federation of Gynecology and Obstetrics; NHE1, Na^+/-H^+ exchanger isofrom 1.
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.

**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 pH (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 micro-environment is hypothesized to accelerate extracellular matrix remodeling, which results in metastasis (22). NHE1, as an isofrom 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 pH and pHe via ion exchange (23).
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.

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  | 1.023 (0.643-1.627)| 0.925    | 1.435 (0.848-2.428)| 0.178    |
| >0                             | 68  | Reference          |          | Reference      |          |
| Serum CA-125, U/ml             |     |                    |          |                |          |
| <35                            | 17  | 0.794 (3.739-23.249)| 0.483    | 1.159 (0.499-2.693) | 0.731    |
| >35                            | 112 | Reference          |          | Reference      |          |
| FIGO stage                     |     |                    |          |                |          |
| I/II                           | 37  | Reference          |          | Reference      |          |
| III/IV                         | 92  | 9.324 (3.739-23.249)| <0.001a | 0.117 (0.045-0.036) | <0.001a |
| Grade                          |     |                    |          |                |          |
| G1                             | 23  | Reference          |          | Reference      |          |
| G2/G3                          | 106 | 14.339 (3.495-58.824)| <0.001a | 0.082 (0.019-0.351) | 0.001a |
| 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)| 0.021a   | 0.594 (0.332-1.063) | 0.079    |
| NHE1 expression                |     |                    |          |                |          |
| Low                            | 27  | Reference          |          | Reference      |          |
| High                           | 102 | 4.212 (1.922-9.230)| <0.001a | 0.402 (0.173-0.993) | 0.034a |

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

1. American Cancer Society: Cancer Facts & Figures. American Cancer Society. Available at http://www.cancer.org/acs/groups/content/@research/documents/document/acspc-047097. Accessed: March 21, 2016.

2. Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, et al: Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. Lancet Oncol 16: 928-936, 2015.

3. Aghajanian C, Goff B, Nycurm LR, Wang YV, Husain A and Blank SV: Final overall survival and safety analysis of OCEANS, a phase 3 trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent ovarian cancer. Gynecol Oncol 139: 10-16, 2015.

4. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, Kurzeder C, et al: A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med 365: 2484-2496, 2011.

5. Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, Sovak MA, Yi J and Nycurm LR: OCEANS: A randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol 30: 2093-2045, 2012.

6. Stockler MR, Hilpert F, Friedlander M, King MT, Wenzel L, Lee CK, Joly F, de Gregorio N, Arranz JA, Mirza MR, et al: Patient-reported outcome results from the open-label phase III AURELLA trial evaluating bevacizumab-containing therapy for platinum-resistant ovarian cancer. J Clin Oncol 32: 1309-1316, 2014.

7. Oza AM, Cibula D, Benzaquen AO, Poole C, Mathijssen RH, Sonke GS, Colombo N, Späcker J, Vuylatpeke P, Hirte H, et al: Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: A randomised phase 2 trial. Lancet Oncol 16: 87-97, 2015.

8. Liu JF, Barry WT, Birrer M, Lee JM, Buckanovich RJ, Fleming GF, RhoA and Rac1 signaling, resulting in motility and invasion in human breast cancer cells. Clin Cancer Res 9: 2366-2373, 2003.

9. Tommasino M, Casavola V and Reshkin SJ: The Na+/H+ exchanger NHE1 mediates cytoskeletal changes involving reciprocal modulation of Na(+) and H(+) extrusion, cell migration and survival. Carcinogenesis 37: 839-851, 2016.

10. Loo SY, Chang MK, Chuas C, Kumar AP, Pervaiz S and Clement MV: NHE-1: A promising target for novel anti-cancer therapeutics. Curr Pharm Des 18: 1372-1382, 2012.

11. Amith SR and Fliegel L: Regulation of the Na(+)/H(+) exchanger (NHE1) in breast cancer metastasis. Cancer Res 73: 1259-1264, 2013.

12. Amith SR, Wilkinson JM, Baksh S and Fliegel L: The Na(+)/H(+) exchanger (NHE1) as a novel co-adjuvant target in paclitaxel therapy of triple-negative breast cancer cells. Oncotarget 6: 1262-1275, 2015.

13. Lin Y, Chang G, Wang J, Jin W, Wang L, Li H, Ma L, Li Q and Pang T: NHE1 mediates MDA-MB-231 cells invasion through the regulation of MT1-MMP. Exp Cell Res 317: 2031-2040, 2011.

14. Yang X, Wang D, Dong W, Song Z and Dou K: Suppression of Na(+)/H(+) exchanger 1 by RNA interference or amiloride inhibits human hepatoma cell line SMMC-7721 cell invasion. Med Oncol 28: 385-390, 2011.

15. Yang X, Wang D, Dong W, Song Z and Dou K: Expression and modulation of Na(+)/H(+) exchanger 1 gene in hepatocellular carcinoma: A potential therapeutic target. J Gastroenterol Hepatol 26: 364-370, 2011.

16. Cardone RA, Greco MR, Zeeberg K, Zaccagnino A, Saccomano M, Bellizzi A, Bruns P, Menga M, Pilarsky C, Schwab A, et al: A novel NHE1-centered signaling cassette drives epidermal growth factor receptor–dependent pancreatic tumor metastasis and is a target for combination therapy. Neoplasia 17: 155-166, 2015.

17. Lin Y, Wang J, Jin W, Wang L, Li H, Ma L, Li Q and Pang T: NHE1 mediates migration and invasion of HeLa cells via regulating the expression and localization of MT1-MMP. Cell Biochem Funct 30: 41-46, 2012.

18. Lin Y, Li Q, Wang J, Chang G, Lin Y, Li H, Wang L, Gao W and Pang T: Na(+)/H(+) exchanger 1 inhibition contributes to K562 leukemic cell differentiation. Cell Biol Int 36: 739-745, 2012.

19. Tang L, Yang J, Ng SK, Rodriguez N, Choi PW, Vitonis A, Wang K, McLachlan GJ, Caiazza RJ Jr, Liu BC, et al: Autoantibody profiling to identify biomarkers of key pathogenic pathways in mucinous ovarian cancer. Eur J Cancer 46: 170-179, 2010.

20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(ΔΔCT) method. Methods 25: 402-408, 2001.

21. Wang H, Mu X, Zhou S, Zhang J, Dai J, Tang L, Xiao L, Duan Z, Jia L and Chen S: NEDD9 overexpression is associated with the progression of and an unfavorable prognosis in epithelial ovarian cancer. Hum Pathol 45: 401-408, 2014.

22. Greco MR, Antelmi E, Busco G, Guerra L, Rubino R, Casavola V, Reshkin SJ and Cardone RA: Protease activity at invadopodial focal digestive areas is dependent on NHE1-driven acidic pHe. Oncol Rep 31: 940-946, 2014.

23. Maley K, Strese K, Kämpfer F, Ueberall F, Baierl G, Gaffa-Tarabiz N, Grunike HH and Lettges M: Critical role of protein kinase C alpha and calcium in growth factor induced activation of the Na(+)/H(+) exchanger NHE1. FEBS Lett 521: 205-210, 2002.

24. Cardone RA, Casavola V and Reshkin SJ: The role of disturbed pH dynamics and the Na(+)/H(+) exchanger in metastasis. Nat Rev Cancer 5: 786-795, 2005.

25. Stock C, Ludwig FT and Schwab A: Is the multifunctional Na(+)/H(+) exchanger isoform 1 a potential therapeutic target in cancer? Curr Med Chem 19: 647-660, 2012.

26. Cong D, Zhu W, Shi Y, Pointer KB, Clark PA, Shen H, Kuo JS, Hu S and Sun D: Upregulation of NHE1 protein expression enables glioblastoma cells to escape TMZ-mediated toxicity via increased H(+) extrusion, cell migration and survival. Carcinogenesis 35: 2014-2024, 2014.

27. Zhu W, Carney KE, Pigott VM, Falgoust LM, Clark PA, Kuo JS and Sun D: Glioma-mediated microglial activation promotes glioma proliferation and migration: Roles of Na(+)/H(+) exchanger isoform 1. Carcinogenesis 37: 839-851, 2016.

28. Paradiso A, Cardone RA, Bellizzi A, Bagorda A, Guerra L, Tommasino M, Casavola V and Reshkin SJ: The Na(+)–H(+) exchanger-I induces cytoskeletal changes involving reciprocal RhoA and Rac1 signaling, resulting in motility and invasion in MDA-MB-435 cells. Breast Cancer Res 6: R66s-R68s, 2004.

29. Reshkin SJ, Bellizzi A, Cardone RA, Tommasino M, Casavola V and Paradiso A: Paclitaxel induces apoptosis via protein kinase A- and p38 mitogen-activated protein-dependent inhibition of the Na(+)/H(+) exchanger (NHE) NHE1 isoform 1 in human breast cancer cells. Clin Cancer Res 9: 2366-2373, 2003.

30. Aiyoshi Y, Shiozaki A, Ichikawa D, Shimizu H, Kosuga T, Konishi H, Komatsu S, Fujisawa H, Okamoto K, Kishimoto M, et al: Na(+)/H(+) exchanger 1 has tumor suppressive activity and prognostic value in esophageal squamous cell carcinoma. Oncotarget 8: 2209-2223, 2017.

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