Novel regulatory program for norepinephrine-induced epithelial–mesenchymal transition in gastric adenocarcinoma cell lines

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Norepinephrine and epinephrine, catecholamine hormones that are major mediators for chronic stress-induced cancers, are implicated in the progression of a number of cancer cells, including gastric adenocarcinoma. However, the underlying mechanisms of these hormones have not been well elucidated. Epithelial–mesenchymal transition (EMT) is a crucial event responsible for cancer cell invasion and metastasis. The hypothesis regarding whether the promotive effects of norepinephrine (NE) on cancer are in part due to its ability to induce an EMT program has not been proven. In this study, we show that NE does not only obviously induce EMT alterations in the morphological characteristics of gastric adenocarcinoma cells, but also increases the markers of EMT, including vimentin expression, and decreases E-cadherin expression, further resulting in cell motility and invasiveness. We also reveal that these actions are mainly mediated through the activation of β_2-AR–HIF-1α–Snail signaling pathways. In summary, this study implies that NE induces EMT in gastric adenocarcinoma through the regulation of β_2-AR–HIF-1α–Snail activity. The data provide a new perspective on chronic stress in a negative social and psychological state, which may be a risk factor for cancer development and progression.

Gastric adenocarcinoma is the second leading cause of cancer-related deaths.[1] Epidemiological data show that chronic stress in a negative social and psychological state such as depression may be a risk factor for cancer development and progression.[2–4] Underlying mechanistic studies have identified that response to stressors can activate the hypothalamic–pituitary–adrenal axis. Such activation leads to the release of catecholamines from the adrenal gland as well as from the brain and sympathetic nerve terminals. These hormones not only affect cellular immune function, but also contribute directly to tumor growth, migration, and invasive capacity, and angiogenesis through the biological signaling pathways.[5–7]

The role of catecholamines (NE and epinephrine) has been increasingly recognized among these stress hormones. The pro-cancer effect of catecholamines is primarily mediated by β_2-adrenergic receptor (β_2-AR), which stimulates the signaling cascade through adenylyl cyclase and its downstream effectors.[8] Evidence also indicates that β_2-AR controls mitogenic and/or anti-apoptotic signaling activation in the adenocarcinomas of the lungs, prostate, colon, and ovary.[8–12] Recently, *in vivo* experiment data from stress animal models that use ovarian carcinoma provided compelling evidence that catecholamines may directly modulate the growth and malignant behavior of tumors independent of the effects on the immune system.[13] In a previous study that used constraint stress and a terrifying noise stress model in a pancreatic cancer xenograft model, we showed that chronic stress can promote tumor progression through the β_2-AR–hypoxia-inducible factor-1α (HIF-1α) regulatory axis.[14] However, it was not established whether or not epithelial–mesenchymal transition (EMT) is responsible for stress-induced tumor invasion.

Epithelial–mesenchymal transition is regarded as a pivotal event in the initial step of a metastatic cascade.[15] During the EMT of *in situ* cancer cells, epithelial cell layers lose polarity together with cell–cell contacts. These layers then undergo a dramatic remodeling of the cytoskeleton, resulting in enhanced cell migration and invasion ability. After migrating to the suitable site, tumor cells re-express epithelial markers through "mesenchymal–epithelial transition".[16,17] The induction of EMT is driven through the complex interplay between tumor environment and cancer cells. The mechanisms include the activation of several transcriptional repressors, notably, Snail, Slug, and Twist, through multiple cellular signaling pathways, such as nuclear factor-κB, Wnt, and Hedgehog.[18,19] Thus, reversing or blocking EMT is a promising therapeutic strategy to limit cancer diffusion.

Norepinephrine can induce cancer cell invasion through the β_2-AR–HIF-1α regulatory axis, where HIF-1α is critically involved in the acquisition of Snail-mediated EMT. During progression to metastatic competence, carcinoma cells enter an EMT program that allows them to acquire the features of mesenchymal-like cells that may significantly endow invasiveness. Whether chronic stress-induced tumor invasiveness mediated by NE can be partially due to EMT

has not been confirmed. The goal of this study is to investigate the effects of NE on the EMT program of gastric adenocarcinoma cells.

Materials and Methods

Cell cultures and treatments. Human gastric adenocarcinoma cell lines BGC-823 and SGC-7901 (obtained from ATCC, Manassas, VA, USA) were maintained in DMEM (Gibco BRL, Gaithersburg, MD, USA) supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), 0.1 mM non-essential amino acids, 0.2 mM glutamine, 1 mM pyruvate, and 10% heat-inactivated FBS, and then incubated in 5% CO_2 humidified atmosphere at 37°C. Cells were grown to 80% confluency prior to treatment. The antibodies against HIF-1α, Snail, E-cadherin, vimentin, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The β$_2$-AR antagonist, ICI 118551, and the HIF-1α inhibitor, 2-methoxyestradiol, were purchased from Sigma Chemical Co. Ltd (St. Louis, MO, USA).

Scanning electron microscopy. The cells treated or untreated with NE were harvested and rinsed with PBS. Cells were fixed for 2 h in 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M PBS (pH 7.4), rinsed again in PBS, and post-fixed in 1% osmium tetroxide for 1 h. After washing in PBS, the cells were progressively dehydrated in a 10% graded series of 30–100% ethanol, and then dried in 70–100% acetonitrile solution. Finally, the cells were sprayed with gold and examined under a scanning electron microscope.

Cell invasion assay. Cell invasion assay was carried out and evaluated as described in detail by using Boyden chambers equipped with 8-μm porosity polyvinylpyrrolidone-free polycarbonate filters coated with 50 μg/mL Matrigel solution. The cells were first seeded in 12-well plates at a concentration of 2.5×10^5 per well and cultured for 48 h with NE (10 μM). Normal culture medium was added at the bottom chamber to induce the cancer cell lines. Pretreated cells were seeded in the top chamber. The Matrigel invasion chamber was incubated for 24 h in a humidified tissue culture incubator. After 24 h, the non-invasive cells were removed from the upper surface of the separating membrane by gentle scrubbing with a cotton swab. The invading cells were fixed in 100% methanol, stained with 0.1% crystal violet solution, and then counted under a microscope at 200× magnification.

Reverse transcription-PCR and real-time quantitative PCR. Total RNA from the BGC-823 and SGC-7901 cells was isolated using TRIzol reagent (Gibco BRL), and the quantities were determined spectrophotometrically. First-strand cDNA was synthesized from 2 μg total RNA using the RevertAid Kit (Fermentas MBI, Sacramento, CA, USA). The sequences of the PCR primers were as follows: E-cadherin (502 bp) forward 5′-CGC ATT GCC ACA TAC A-3′ and reverse 5′-CGT TAG CCT CGT TCT CA-3′; vimentin (690 bp) forward 5′-CGC TTC GCC AAC TAC AT-3′ and reverse 5′-AGG GCA TCC ACT TCACAG-3′; and β-actin (179 bp) forward 5′-ATC GTG CGT GAC ATT AAG GAG AAG-3′ and reverse 5′-AGG AAG GAA GGC TGG AAG AGT G-3′. The PCR conditions included an initial cDNA synthesis reaction at 42°C for 1 h using the RevertAid Kit (Fermentas MBI), followed by 22 cycles of a denaturation step for 5 min at 94°C, 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. After the last cycle, a final extension was carried out at 72°C for 10 min. The housekeeping gene β-actin was used as an internal control.

Real-time quantitative PCR was carried out with Platinum SYBR Green qPCR SuperMix UDG (Invitrogen, Carlsbad,

CA, USA) using the Rotor-Gene RG-3000 (Corbett Research, Doncaster, Vic., Australia).

Western blot analysis. Briefly, 5×10^5 cells were incubated on ice for 30 min in 0.5 mL ice-cold whole-cell lysate buffer. Debris was removed by centrifugation, and the protein content of the cell was determined. The cellular lysates were separated by 10% SDS-PAGE, and then electrotransferred into nitrocellulose membranes. After being blocked with 5% non-fat milk in TBST, the membranes were incubated with primary antibodies at 4°C overnight, followed by 1:2000 HRP-conjugated secondary antibody (Santa Cruz Biotechnology) for 2 h. Immunoreactive bands were visualized using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The Western blot signals were quantitated by densitometric analysis using Total Lab Nonlinear Dynamic Image analysis software (MathWorks, Natick, MA, USA).

Immunofluorescence assay. Exponentially growing cells were seeded on 25-mm square glass cover slips placed in 35-mm diameter culture dishes. After treatment, the cells were fixed with 4% formaldehyde for 5 min, permeabilized with 0.2% solution of Triton X-100 in PBS, and blocked with 2% BSA-PBS for 30 min. Slides were incubated overnight with CY3-labeled anti-E-cadherin (1:100) and FITC-labeled anti-vimentin (1:100). Cell nuclei were counterstained by DAPI. Fluorescent imaging was obtained with a confocal laser scanning microscope (Carl Zeiss MicroImaging, Jena, Germany).

Small interfering RNA assay. To inhibit the expressions of β$_2$-AR and HIF-1α, we used siRNA oligos (β$_2$-AR siRNA target sequence, 5′-CAGAGTGGATATCACGTGGAA-3′; HIF-1α siRNA target sequence, 5′-AGGAAGAACTATGAACATA AA-3′; Qiagen, Germany, (Shanghai agent in China)). The BGC-823 cells ($n = 2 \times 10^6$) were transfected with siRNA targeted against β$_2$-AR (100 nm/L) or a control siRNA (Qiagen) using Lipofectamine 2000 (Invitrogen). Cells were covered overnight before starvation. This procedure was then followed by treatment with NE (10 μM) for 12 h. Finally, the cells were harvested for RT-PCR and invasion assay.

Statistical analysis. Each experiment was carried out at least three times. Data were shown by their mean values ± standard deviation, and differences were evaluated using Student's t-test and one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

Results

Norepinephrine induced cell morphological changes of EMT in gastric adenocarcinoma cells. To determine whether stress hormones can induce EMT, we initially investigated the cell morphological changes of EMT by using optical and scanning electron microscopy in the gastric adenocarcinoma cells BGC-823 and SGC-7901 exposed to NE (10 μM; this concentration was chosen based on our previous study)[20] treatment. Both cell lines were treated with NE for 48 h. Figure 1 shows that the cancer cells underwent typical EMT morphological changes; specifically, the cells started to lose cell contacts, scattered from cell clusters, and acquired a spindle-shaped, fibroblast-like phenotype according to optical microscopy. Scanning electron microscopy results showed that the extracellular microvilli increased in some cells. These results suggest that NE can induce an occurrence of the EMT process.

Norepinephrine regulated expression of EMT markers in gastric adenocarcinoma cells. To further confirm the EMT phenomenon, we sequentially tested EMT markers, such as E-cadherin and
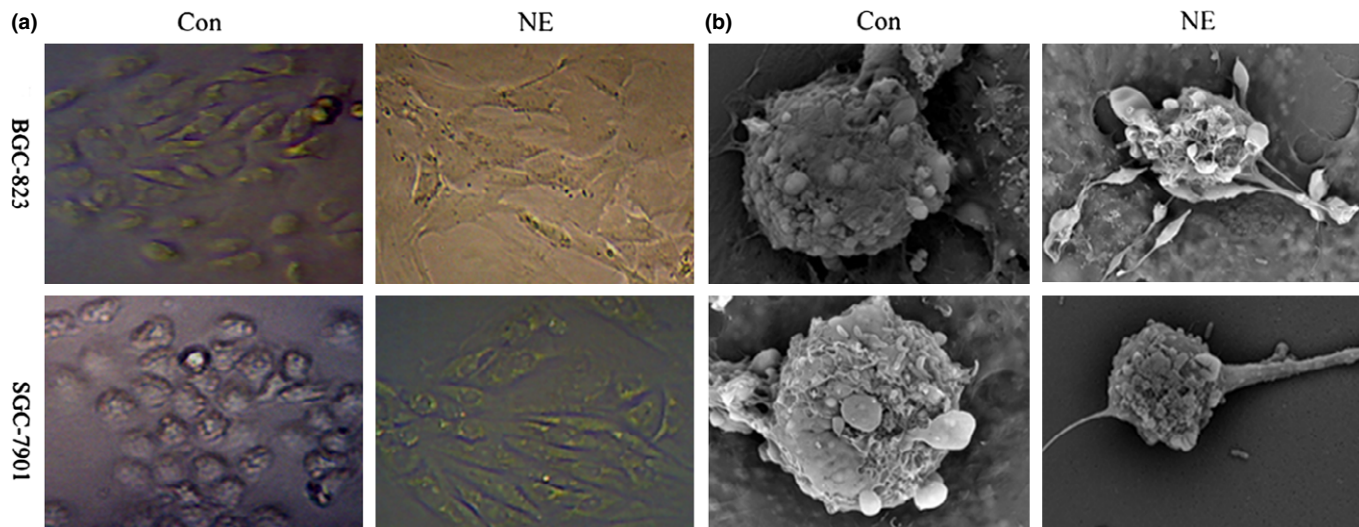
Fig. 1. Stress hormone norepinephrine (NE) induced cell morphological changes of epithelial–mesenchymal transition in gastric adenocarcinoma SGC-7901 and BGC-823 cells. Cells were incubated with either PBS or norepinephrine (10 μM). After 48 h, cellular morphological changes were examined by optical (×400) (a) and scanning electron microscopy (b) (×8000–20 000). Results represent three independent experiments. Con, control.
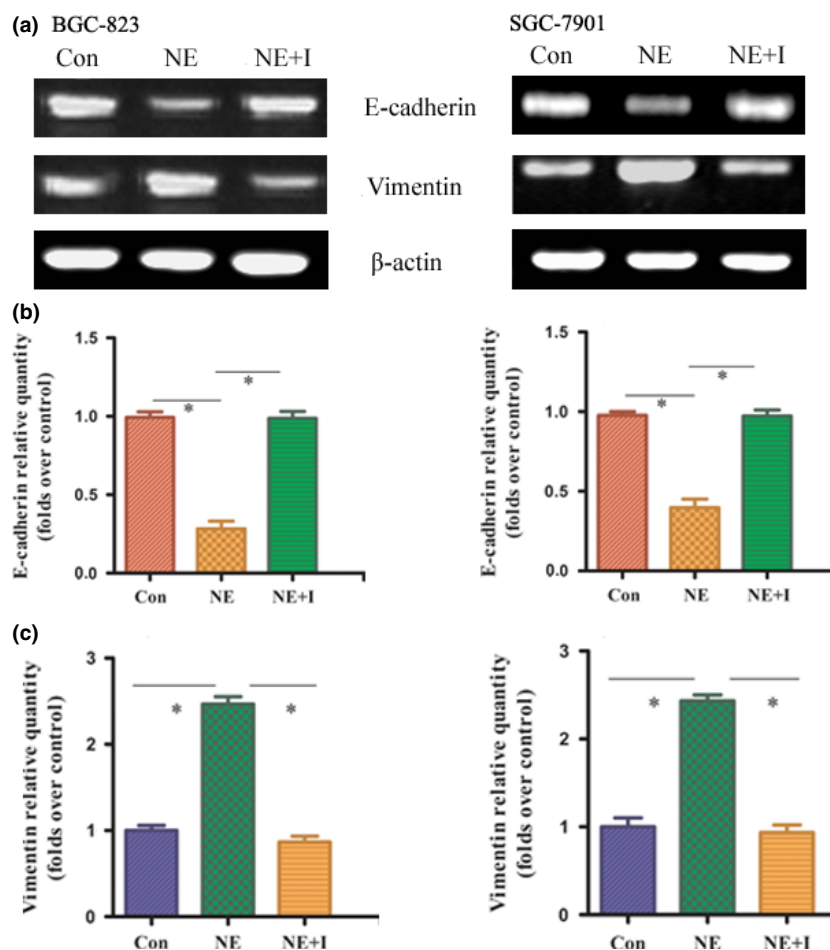

Fig. 2. Norepinephrine (NE) decreased the expression of E-cadherin mRNA and increased the expression of vimentin mRNA in gastric adenocarcinoma cell lines, whereas the β2-adrenergic receptor antagonist ICI 118551 reversed this effect. (a) mRNA expressions of E-cadherin and vimentin in SGC-7901 and BGC-823 cells were determined by RT-PCR. (b, c) Quantification of mRNA by real-time quantitative PCR. Data from at least three independent experiments with duplicate determinations are expressed as means ± SEM. *$P < 0.05$ was considered statistically significant. Con, control; NE+I, NE + ICI 118551.

vimentin, as well as mRNA and protein expressions. The RT-PCR and real-time quantitative PCR analysis (Fig. 2) indicated that the mRNA levels of vimentin and E-cadherin are significantly increased and suppressed by NE, respectively ($P < 0.05$).

However, these effects can be reversed by the β2-AR antagonist ICI 118551. Western blot analysis (Fig. 3) showed that E-cadherin expression is significantly downregulated in the NE group compared with the control, whereas vimentin expression is
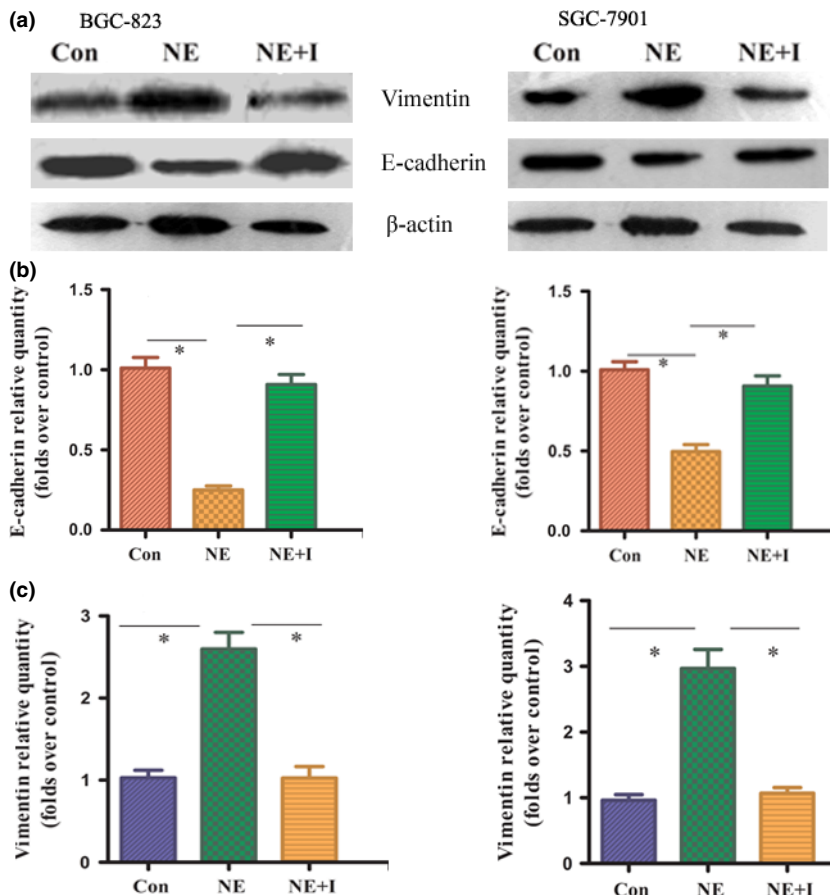
Fig. 3. Norepinephrine (NE) decreased the expression of E-cadherin protein and increased the expression of vimentin protein in gastric adenocarcinoma cell lines, whereas the β_2-adrenergic receptor antagonist ICI 118551 reversed this effect. (a) Protein expressions of E-cadherin and vimentin in SGC-7901 and BGC-823 cells were determined by Western blot analysis. (b, c) Quantification of E-cadherin and vimentin protein. Data from at least three independent experiments with duplicate determinations are expressed as means ± SEM. *$P < 0.05$ was considered statistically significant. Con, control; NE+I, NE + ICI 118551.

substantially increased ($P < 0.05$). We also presented evidence that the β_2-AR antagonist ICI 118551 can reverse NE-induced EMT, which is accompanied by the inhibition of vimentin protein expression and an increase in the expression of E-cadherin protein.

To further determine possible alterations in E-cadherin and vimentin, BGC-823 and SGC-7901 cells treated with NE underwent fluorescence immunostaining and were then analyzed by confocal microscopy. Results after 48 h indicated that the E-cadherin fluorescence signal in the NE group was lower than in the control group, whereas the vimentin fluorescence signal was substantially increased (Fig. 4). These results further suggest that NE has promotive effects on cellular EMT.

Hypoxia-inducible factor-1α–Snail signaling is a key factor to decrease E-cadherin and increase vimentin expression. As reported in a previous study,[14] NE can increase cancer cell invasion through the β_2-AR–HIF-1α regulatory axis. Hypoxia-inducible factor-1α is also critically involved in the acquisition of EMT mediated by the direct downstream transcription factor Snail, which is also an upstream control gene of E-cadherin and vimentin. To explore whether the EMT effect of NE is associated with the activation of the β_2-AR–HIF-1α–Snail regulatory axis, the expressions of HIF-1α and Snail protein were detected in gastric adenocarcinoma cells by Western blot analysis. Our results showed that NE promotes HIF-1α and Snail protein expressions, which are accompanied by the decrease of E-cadherin and the increase of vimentin (Fig. 5). We further presented evidence that the β_2-AR antagonist ICI 118551 can also reverse NE-induced HIF-1α and

Snail protein expressions. These results indicated that β_2-AR-HIF-1α-Snail has a critical role in EMT.

β_2-Adrenergic receptor is required to induce EMT phenomenon for NE. To further evaluate whether β_2-AR activation induced by NE is essential for EMT to occur in gastric adenocarcinoma cells, the effects of β_2-AR siRNA on the EMT of BGC-823 gastric adenocarcinoma cells were examined. BGC-823 was chosen because its cell line shows a higher expression of β_2-AR. The efficacy of β_2-AR siRNA to knockdown β_2-AR mRNA and protein was confirmed by RT-PCR and Western blot, respectively. We observed that both β_2-AR mRNA and protein levels (Fig. 6a,b) were barely detectable in β_2-AR siRNA-transfected cells compared with control siRNA-transfected cells. Subsequently, NE did not induce the EMT phenomenon (Fig. 6c,d). Moreover, after treatment with NE in transfected cells, the expressions of HIF-1α, Snail, and vimentin were attenuated to a much greater extent than those of the control cells, whereas E-cadherin showed an opposite alteration (Fig. 6e,f). The results showed the critical effects of β_2-AR activity in NE on the EMT of cancer cells.

Hypoxia-inducible factor-1α linked β_2-AR–Snail regulatory axis to induce EMT phenomenon for NE. To further evaluate whether HIF-1α induced by NE can be a hub for the occurrence of EMT in gastric adenocarcinoma cells, the effects of HIF-1α siRNA on the EMT of gastric adenocarcinoma cell line BGC-823 were examined. The efficacy of HIF-1α siRNA to knockdown HIF-1α mRNA and protein was confirmed by RT-PCR and Western blot, respectively. We observed that both HIF-1α mRNA and protein levels (Fig. 7a,b) were significantly lower in HIF-1α siRNA-transfected cells than in control
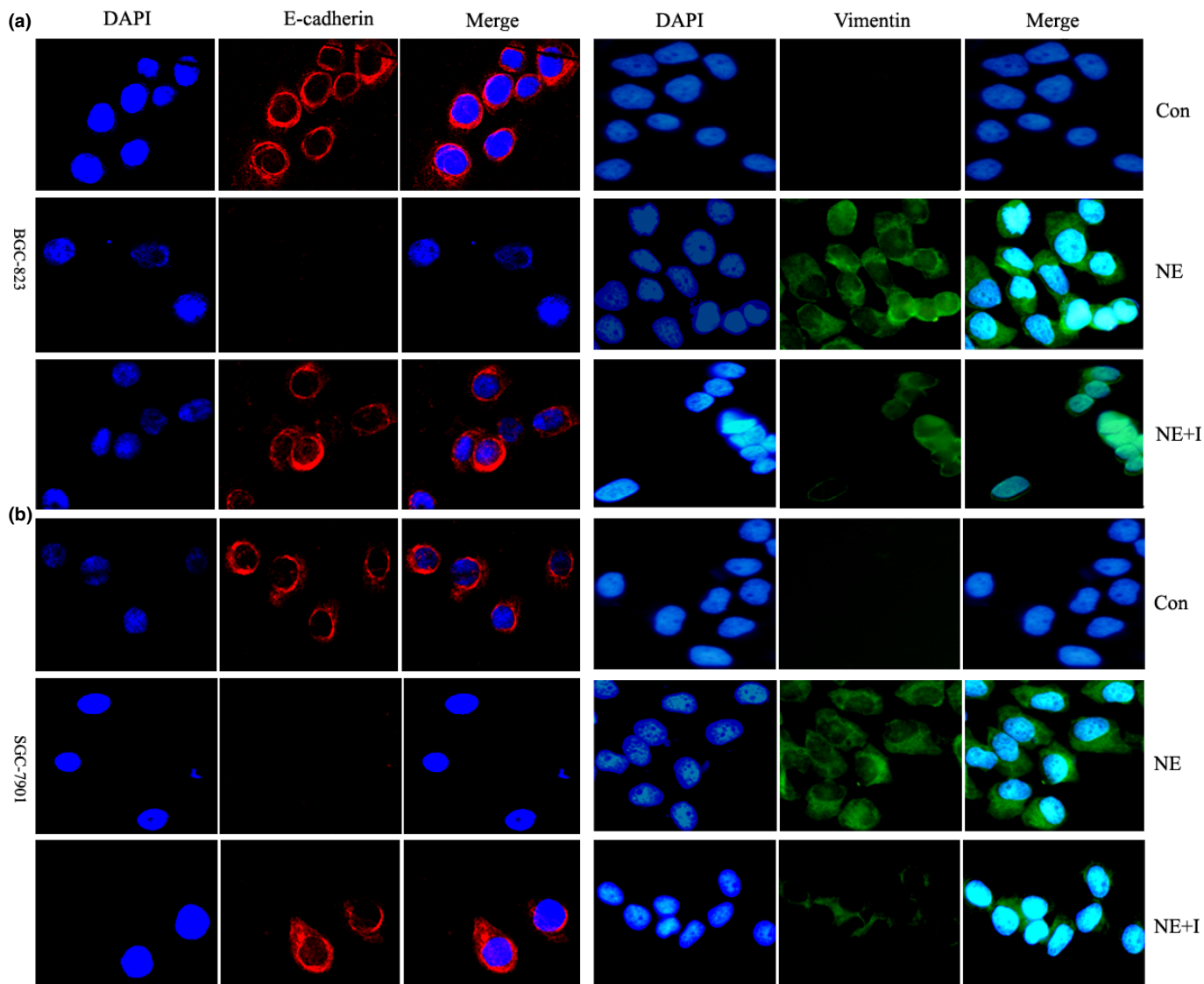
Fig. 4. Immunodetection of E-cadherin and vimentin proteins in gastric adenocarcinoma cell lines. BGC-823 (a) and SGC-7901 (b) cells were incubated with norepinephrine (NE, 10 μM). After 48 h, fluorescent imaging was obtained with a confocal laser scanning microscope. E-cadherin fluorescence signal in the norepinephrine group is lower than in the control (Con) group, whereas vimentin is higher in the control group. NE + I, NE + ICI 118551.

siRNA-transfected cells. Subsequently, NE did not induce the EMT phenomenon (Fig. 7c,d). After treatment with NE in transfected cells, the expressions of Snail and vimentin were also attenuated to a much greater extent than those of the control cells, whereas E-cadherin showed an opposite alteration (Fig. 7e,f). The similar results showed the hub role of HIF-1α in the β2-AR–Snail regulatory mechanism of cancer cell EMT.

Norepinephrine increased invasiveness of gastric adenocarcinoma cells through β2-AR. Epithelial–mesenchymal transition is associated with enhanced cellular progression. Our observation that NE induced EMT prompted us to examine whether NE affects the invasion of gastric adenocarcinoma cells. The motile phenotype of NE-treated cells was evaluated by invasion assay. The number of invasive cells treated with NE significantly increased compared with that in the control group. After treatment in the β2-AR siRNA-transfected cells group, the results showed that NE did not increase the number of invaded cancer cells (Fig. 8a,b); however, it accompanied changes in E-cadherin and vimentin expression (Fig. 8c,d). These results

suggested that β2-AR mediates the stimulative invasion of NE in gastric adenocarcinoma cells.

Discussion

Long exposure to a psychological distress is considered to be a key factor in the etiology of numerous diseases.[21,22] Although its underlying mechanism has not been well elucidated, the role of catecholamine (NE and epinephrine) release induced by stressors has been increasingly recognized. Epithelial–mesenchymal transition is a crucial event responsible for cancer cell invasion and metastasis.[23–25] However, the contribution of EMT to the tumor-promoting effect of chronic stress is unknown. In this study, we showed that BGC-823 and SGC-7901 cells can be induced by NE to undergo EMT, and that β2-AR–HIF-1α–Snail signaling is required for this phenomenon to occur. These findings enhance our understanding of the function and mechanism of chronic stress in cancer.
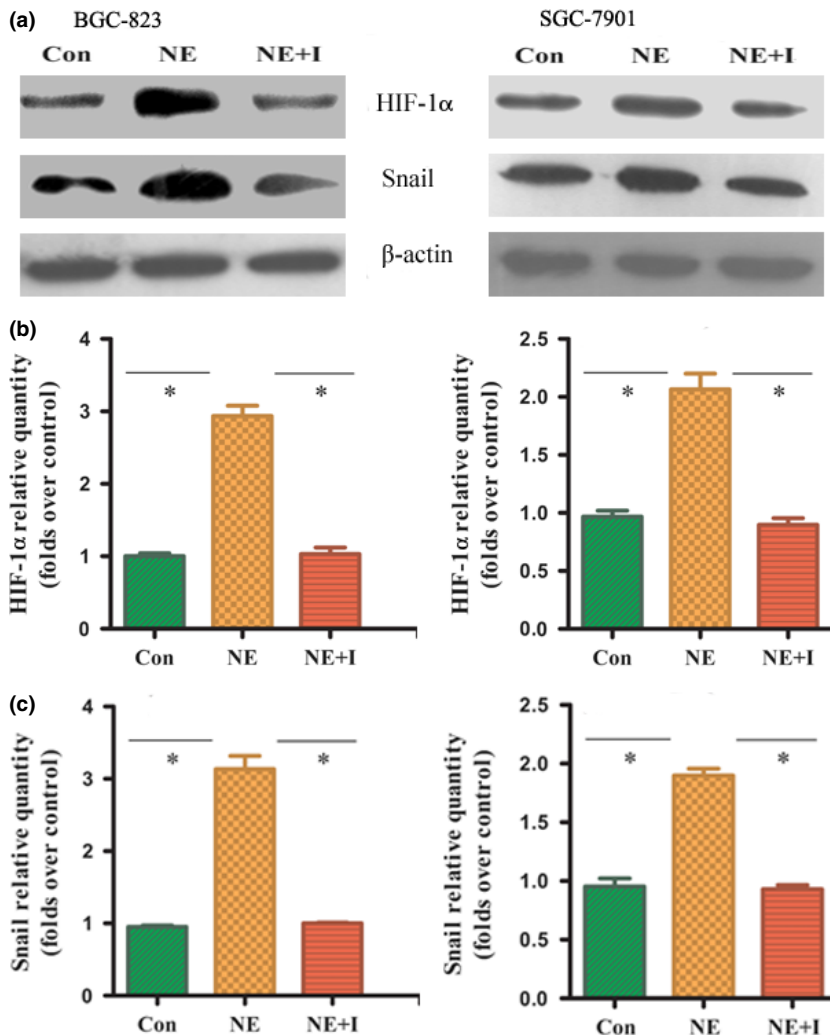
Fig. 5. Norepinephrine (NE) increased the expressions of hypoxia-inducible factor-1α (HIF-1α) and Snail protein in gastric adenocarcinoma cells, whereas the β_2-adrenergic receptor antagonist ICI 118551 reversed this effect. (a) Protein expressions of HIF-1α and Snail in SGC-7901 and BGC-823 cells were determined by Western blot analysis. (b, c) Quantification of HIF-1α and Snail protein. Data from at least three independent experiments with duplicate determinations are expressed as means ± SEM. *$P < 0.05$ was considered statistically significant. Con, control; NE+I, NE + ICI 118551.

Stress pervades almost all aspects of life and is particularly salient during diagnosis, treatment, and follow-up for cancer. In Asian countries like China, doctors do not usually tell their patients directly about their cancer diagnosis because the major concern of doctors is that cancer is a fatal disease that may stress a patient and thus worsen the cancer status. Gomes *et al.* reported that social isolation is associated with elevated tumor NE in ovarian carcinoma patients, a finding that has implications for patient outcomes in ovarian cancer owing to the NE level. [26] Our results further verified the likely mechanism through an *in vitro* experiment. The findings revealed the importance of monitoring emotional distress instead of only monitoring "traditional" vital signs such as blood pressure or heart rate. Yoo *et al.*[27] described emotional distress as "the sixth vital sign in cancer care," and accordingly requested health care providers to offer emotional support. Patients may benefit from the early recognition and adequate treatment of emotional burden, or even depression, as documented in several studies. In addition, the present study presented new scientific evidence of the effect of chronic stress hormones on cancer. It verified the direct contribution of NE to cancer cell invasion through the EMT process, which further facilitated the influence of the β_2-AR–HIF-1α regulatory axis on stress-induced pancreatic tumor progression. Most importantly, our study is the first to show the pro-metastatic effects of the stress hormone NE, which is associated with EMT in cultured gastric adenocarcinoma cells. Our results indirectly offer a new perspective on the role of psychological distress in promoting cancer progression. Meanwhile, the agent of aim to the NE target may prove to be a novel candidate for use in the treatment of carcinoma (especially for patients who are subject to chronic stress due to their cancer diagnosis).

The EMT program is proposed to be a key process during embryonic development and cancer progression.[26] During EMT, epithelial cells acquire mesenchymal, fibroblast-like phenotypes. Epithelial–mesenchymal transition facilitates tumor cell migration from the site of origin and dissemination to distant tissues. This process is triggered by autocrine and paracrine signals. Hypoxia-inducible factor-1α helps to promote and maintain an invasive phenotype.[27] Numerous lines of evidence also identify HIF-1α as an essential central mediator of EMT.[28] For example, studies showed that Snail transcription, which is well established to have a critical role in EMT, is directly activated by HIF-1α.[29] In addition, HIF-1α is identified as the upstream regulator of Twist expression during the EMT of cancer cells.[30,31] Specifically, the induction of Snail mRNA levels during EMT can be reversed by the inhibition of the HIF-1α signaling pathway.[32] Our present
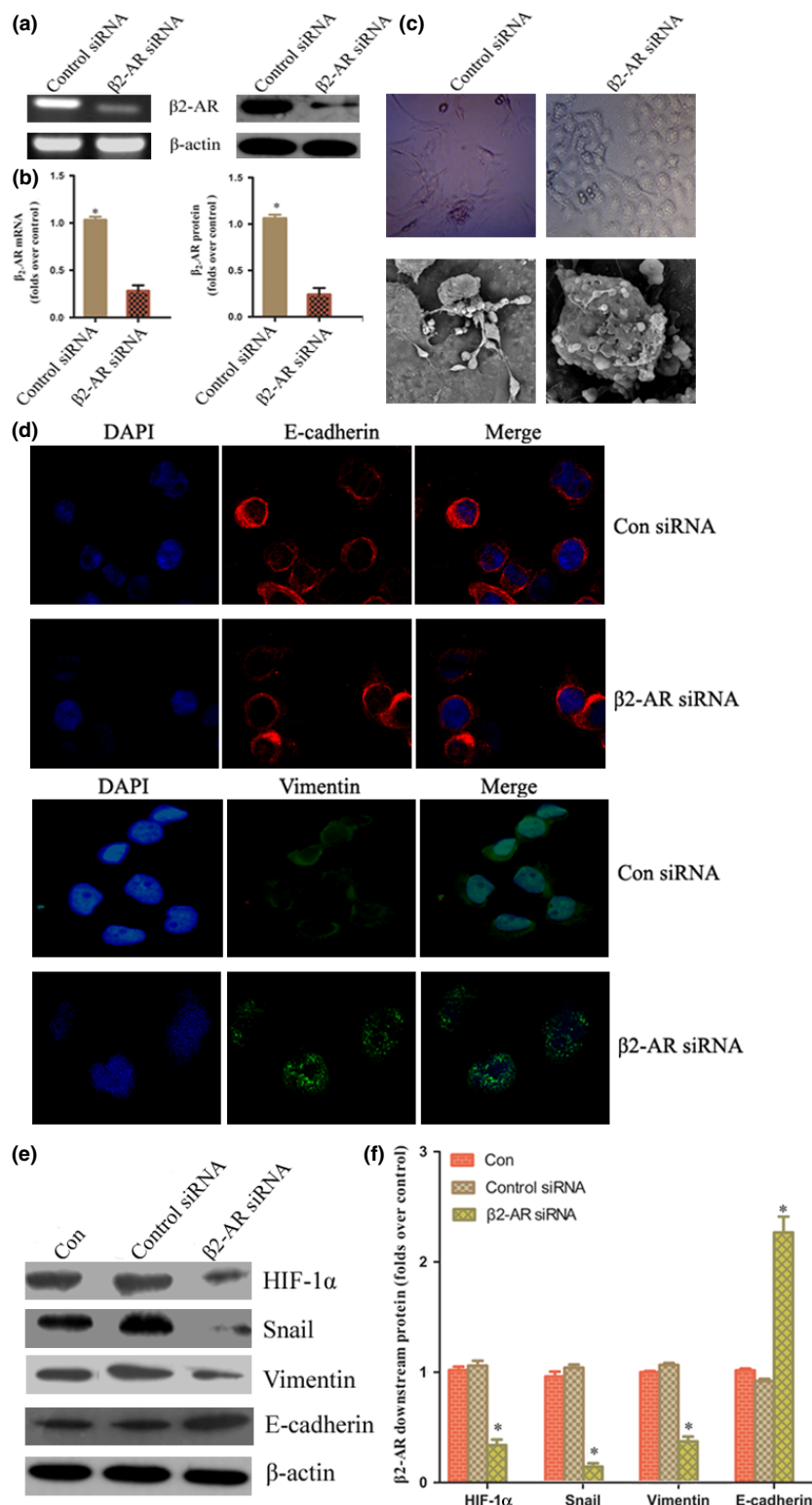
Fig. 6. β2-Adrenergic receptor (β2-AR) siRNA could inhibit norepinephrine (NE)-induced epithelial–mesenchymal transition in gastric adenocarcinoma cells. (a) Efficacy of β2-AR siRNA for knockdown of β2-AR mRNA and protein was confirmed by RT-PCR and Western blot. (b) Quantification of β2-AR mRNA and protein. (c) NE induced cell morphological changes of epithelial–mesenchymal transition in SGC-7901 and BGC-823 cells after β2-AR siRNA. (d) E-cadherin and vimentin fluorescent imaging obtained by a confocal laser scanning microscope showed no disparity between groups. (e) BGC-823 was treated with NE (10 μM) with or without β2-AR siRNA. After 48 h, hypoxia-inducible factor-1α (HIF-1α), Snail, E-cadherin, and vimentin proteins were detected by Western blot analysis. (f) Quantification of HIF-1α, Snail, E-cadherin, and vimentin proteins. *$P < 0.05$ was considered statistically significant. Con, control.

study yields similar results and supports previous findings that HIF-1α is the upstream regulator of Snail and indirectly mediates EMT occurrence, which also reflects a new theory basic of NE to mediate EMT phenomenon through HIF-1α signaling pathway.

E-cadherin is a key factor in the cell–cell adhesion of epithelial cells and acts as a metastatic suppressor in epithelial carcinomas.[33,34] E-cadherin loss is significantly associated with advanced diseases. Vimentin is the major intermediate filament protein found in mesenchymal cells. Vimentin expression is described as the end-stage progression in EMT, representing the completely dedifferentiated state in highly proliferative and invasive tumor cells.[35] Studies revealed that these two important EMT markers are directly regulated by
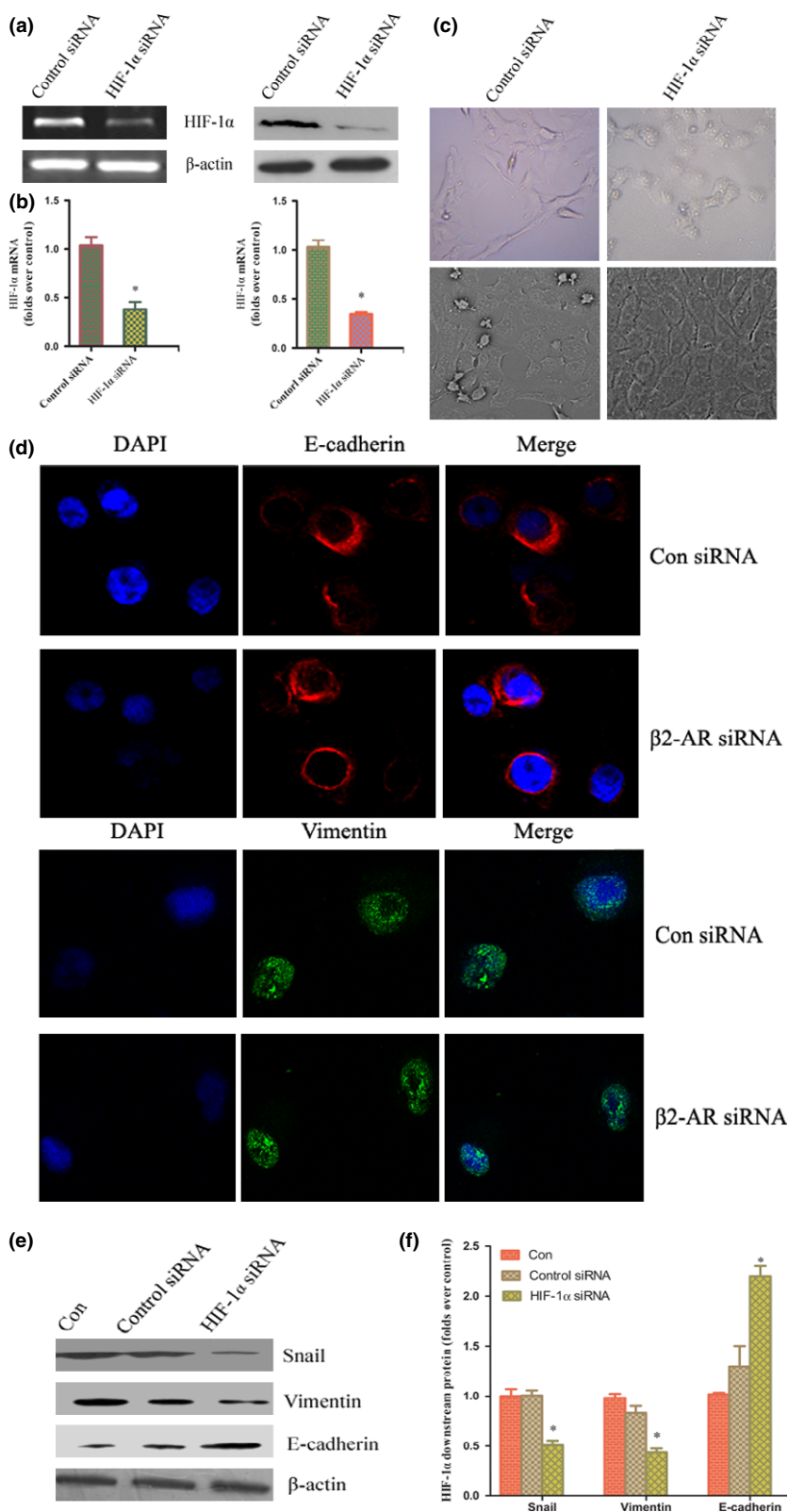
Fig. 7. Hypoxia-inducible factor-1α (HIF-1α) siRNA could inhibit norepinephrine (NE)-induced epithelial–mesenchymal transition in gastric adenocarcinoma cells. (a) Efficacy of HIF-1α siRNA for knockdown of HIF-1α mRNA and protein was confirmed by RT-PCR and Western blot analysis. (b) Quantification of HIF-1α mRNA and protein. (c) NE induced cell morphological changes of epithelial–mesenchymal transition in SGC-7901 and BGC-823 cells after HIF-1α siRNA. (d) E-cadherin and vimentin fluorescent imaging obtained with a confocal laser scanning microscope showed no disparity between groups. (e) BGC-823 was treated with NE (10 μM) with or without HIF-1α siRNA. After 48 h, Snail, E-cadherin, and vimentin proteins were detected by Western blot. (f) Quantification of Snail, E-cadherin, and vimentin proteins. *$P < 0.05$ was considered statistically significant.

Snail. In the present study, we observed that the stress hormone NE can induce EMT if accompanied by E-cadherin loss and vimentin augmentation. Vimentin expression in tumor tissues from mice within the two stress groups is higher than that from mice in the control group, whereas E-cadherin expression is lower. This finding suggests that chronic stress promotes tumor metastasis that is likely associated with EMT. Although the EMT markers are primarily altered because of chronic stress, further study is required to clarify the regulatory mechanism *in vivo*.
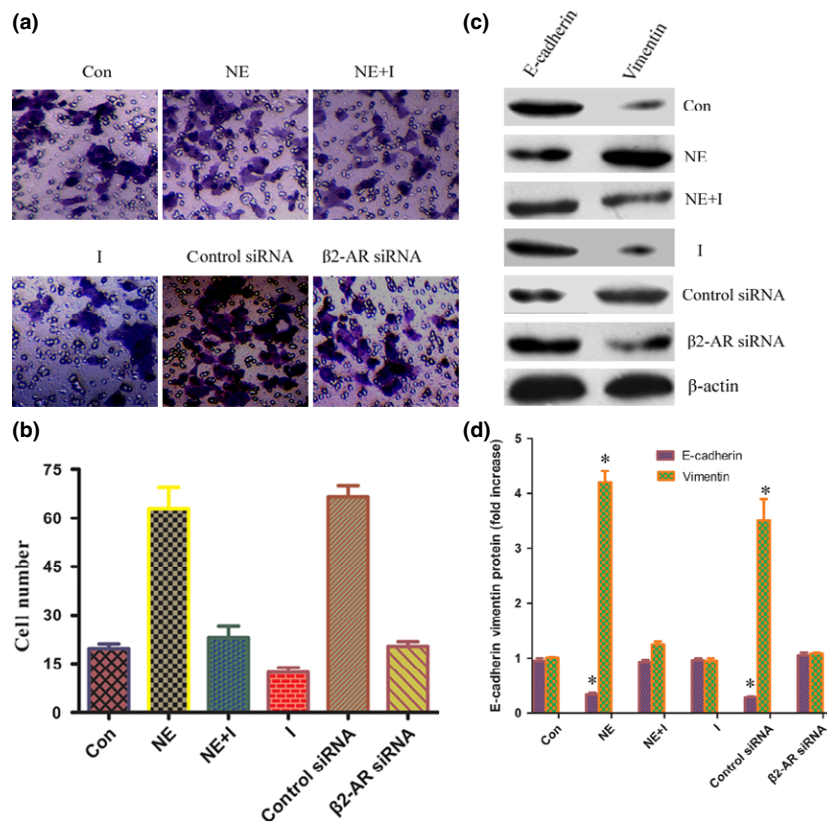
Fig. 8. Cell invasion assay in BGC-823 gastric adenocarcinoma cells. Norepinephrine (NE) significantly ($P < 0.05$) stimulated cell invasion, an effect completely blocked by ICI 118551 or β_2-adrenergic receptor (β_2-AR) siRNA. The photograph shows the bottom side of the filter inserts with cells that have migrated through the filter pores. Columns in the graph represent the count analysis. Con, control; I, ICI 118551.

Taken together, the results of this pioneering study imply that the catecholamine hormone NE induces EMT in gastric adenocarcinoma through the regulation of β_2-AR–HIF-1α–Snail activity. The data provide a new perspective on chronic stress in a negative social and psychological state, which may be a risk factor for cancer development and progression.

Acknowledgments

Disclosure Statement

The authors have no conflict of interest.

References

1 Amin S, Lucas AL, Frucht H. Evidence for treatment and survival disparities by age in pancreatic adenocarcinoma: a population-based analysis. *Pancreas* 2012; **42**: 249–53.

2 Moussas GI, Papadopoulou AG, Christodoulaki AG, Karkanias AP. Psychological and psychiatric problems in cancer patients: relationship to the localization of the disease. *Psychiatrike* 2012; **23**: 46–60.

3 Ng BHP, Tsang HWH. Psychophysiological outcomes of health qigong for chronic conditions: a systematic review. *Psychophysiology* 2009; **46**: 257–69.

4 Bulli F, Miccinesi G, Maruelli A, Katz M, Paci E. The measure of psychological distress in cancer patients: the use of distress thermometer in the oncological rehabilitation center of florence. *Support Care Cancer* 2009; **17**: 771–9.

5 Taylor CB, Conrad A, Wilhelm FH *et al.* Psychophysiological and cortisol responses to psychological stress in depressed and nondepressed older men and women with elevated cardiovascular disease risk. *Psychosom Med* 2006; **68**: 538–46.

6 Spiegel D, Giese-Davis J. Depression and cancer: mechanisms and disease progression. *Biol Psychiatry* 2003; **54**: 269–82.

7 Spiegel D, Sephton SE. Psychoneuroimmune and endocrine pathways in cancer: effects of stress and support. *Semin Clin Neuropsychiatry* 2001; **6**: 252–65.

8 Lin X, Luo K, Lv Z, Huang J. Beta-adrenoceptor action on pancreatic cancer cell proliferation and tumor growth in mice. *Hepatogastroenterology* 2012; **59**: 584–8.

9 Shah SM, Carey IM, Owen CG, Harris T, Dewilde S, Cook DG. Does beta-adrenoceptor blocker therapy improve cancer survival? Findings from a population-based retrospective cohort study. *Br J Clin Pharmacol* 2011; **72**: 157–61.

10 Liao X, Che X, Zhao W, Zhang D, Bi T, Wang G. The beta-adrenoceptor antagonist, propranolol, induces human gastric cancer cell apoptosis and cell cycle arrest via inhibiting nuclear factor kappaB signaling. *Oncol Rep* 2010; **24**: 1669–76.

11 Slotkin TA, Zhang JA, Dancel R, Garcia SJ, Willis C, Seidler FJ. beta-adrenoceptor signaling and its control of cell replication in MDA-MB-231 human breast cancer cells. *Breast Cancer Res Treat* 2000; **60**: 153–66.

12 Fitzgerald PJ. Is norepinephrine an etiological factor in some types of cancer? *Int J Cancer* 2009; **124**: 257–63.

13 Thaker PH, Han LY, Kamat AA *et al.* Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med* 2006; **12**: 939–44.

14 Hu HT, Ma QY, Zhang D *et al.* HIF-1 alpha links beta-adrenoceptor agonists and pancreatic cancer cells under normoxic condition. *Acta Pharmacol Sin* 2010; **31**: 102–10.

15 Jiang J, Tang YL, Liang XH. EMT: a new vision of hypoxia promoting cancer progression. *Cancer Biol Ther* 2011; **11**: 714–23.

16 Ksiazkiewicz M, Markiewicz A, Zaczek AJ. Epithelial-mesenchymal transition: a hallmark in metastasis formation linking circulating tumor cells and cancer stem cells. *Pathobiology* 2012; **79**: 195–208.

17 Tiwari N, Gheldof A, Tatari M, Christofori G. EMT as the ultimate survival mechanism of cancer cells. *Semin Cancer Biol* 2012; **22**: 194–207.

18 Heldin CH, Vanlandewijck M, Moustakas A. Regulation of EMT by TGFbeta in cancer. *FEBS Lett* 2012; **586**: 1959–70.

19 Foroni C, Broggini M, Generali D, Damia G. Epithelial-mesenchymal transition and breast cancer: role, molecular mechanisms and clinical impact. *Cancer Treat Rev* 2012; **38**: 689–97.

20 Guo K, Ma Q, Wang L *et al.* Norepinephrine-induced invasion by pancreatic cancer cells is inhibited by propranolol. *Oncol Rep* 2009; **22**: 825–30.

21 Lemogne C, Consoli SM. Depression and cancer: challenging the myth through epidemiology. *Psycho-oncologie* 2010; **4**: 22–7.

22 Durdux C. Depression and cancer: the point of view of the oncologist. *Psycho-oncologie* 2010; **4**: 36–41.

23 Bastid J. EMT in carcinoma progression and dissemination: facts, unanswered questions, and clinical considerations. *Cancer Metastasis Rev* 2012; **31**: 277–83.

24 Krantz SB, Shields MA, Dangi-Garimella S, Munshi HG, Bentrem DJ. Contribution of epithelial-to-mesenchymal transition and cancer stem cells to pancreatic cancer progression. *J Surg Res* 2012; **173**: 105–12.

25 Zhao R, Wu Z, Zhou Q. Epithelial-mesenchymal transition and tumor metastasis. *Zhongguo Fei Ai Za Zhi* 2011; **14**: 620–4.

26 Gomes LR, Terra LF, Sogayar MC, Labriola L. Epithelial-mesenchymal transition: implications in cancer progression and metastasis. *Curr Pharm Biotechnol* 2011; **12**: 1881–90.

27 Yoo YG, Christensen J, Huang LE. HIF-1alpha confers aggressive malignant traits on human tumor cells independent of its canonical transcriptional function. *Cancer Res* 2011; **71**: 1244–52.

28 Shin HW, Cho K, Kim DW *et al.* Hypoxia-inducible factor 1 mediates nasal polypogenesis by inducing epithelial-to-mesenchymal transition. *Am J Respir Crit Care Med* 2012; **185**: 944–54.

29 Hung JJ, Yang MH, Hsu HS, Hsu WH, Liu JS, Wu KJ. Prognostic significance of hypoxia-inducible factor-1alpha, TWIST1 and Snail expression in resectable non-small cell lung cancer. *Thorax* 2009; **64**: 1082–9.

30 Stefania C, Erica N, Alessandra C *et al.* Redox mechanisms switch on hypoxia-dependent epithelial–mesenchymal transition in cancer cells. *Carcinogenesis* 2008; **29**: 2267–78.

31 Haase VH. Oxygen regulates epithelial-to-mesenchymal transition: insights into molecular mechanisms and relevance to disease. *Kidney Int* 2009; **76**: 492–9.

32 Zhao JH, Luo Y, Jiang YG, He DL, Wu CT. Knockdown of beta-Catenin through shRNA cause a reversal of EMT and metastatic phenotypes induced by HIF-1alpha. *Cancer Invest* 2011; **29**: 377–82.

33 David JM, Rajasekaran AK. Dishonorable discharge: the oncogenic roles of cleaved E-cadherin fragments. *Cancer Res* 2012; **72**: 2917–23.

34 Vered M, Allon I, Buchner A, Dayan D. E-cadherin in oral SCC: an analysis of the confusing literature and new insights related to its immunohistochemical expression. *Histol Histopathol* 2012; **27**: 141–50.

35 Lian N, Lin T, Liu W *et al.* TGFbeta suppresses osteoblast differentiation via the vimentin-activating transcription factor 4 (atf4) axis. *J Biol Chem* 2012; **287**: 35975–84.