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

Tao Shan,1 Xijuan Cui,2 Wei Li,3 Wanrun Lin,4 Yiming Li,1 Xi Chen1 and Tao Wu1

1Department of General Surgery, Second Affiliated Hospital of Medical College, Xi’an Jiaotong University, Xi’an; 2Department of General Surgery, First Affiliated Hospital of Medical College, Xi’an Jiaotong University, Xi’an; 3Graduate School, Fourth Military Medical University, Xi’an; 4Department of Pathology, Shandong Provincial Hospital, Ji’nan, China

Key words
β2-AR, EMT, gastric adenocarcinoma, HIF-1α, norepinephrine

Correspondence Tao Shan, Department of General Surgery, Second Affiliated Hospital of Medical College, Xi’an Jiaotong University, Xi’an Jiaotong University, Xi’an, 710004, China. Tel.: +86-2984501705; Fax: +86-2987679246; E-mail: shantao820304@163.com

Funding information
Scientific Grant of Shaanxi (ky201135), China.

Received February 6, 2014; Revised April 10, 2014; Accepted May 5, 2014

Cancer Sci 105 (2014) 847–856
doi: 10.1111/cas.12438

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 β2-adrenergic receptor (β2-AR), which stimulates the signaling cascade through adenyl cyclase and its downstream effectors.8 Evidence also indicates that β2-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 β2-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 β2-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

© 2014 The Authors. Cancer Science published by Wiley Publishing Asia Pty Ltd on behalf of Japanese Cancer Association.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
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% CO2 humidified atmosphere at 37°C. Cells were grown to 80% confluence 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 β2-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 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 coated with 50 μg/mL of 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'-TCACAG-3'; 5'-ACGAGTTGATATCAGCTGGAA-3'; HIF-1α siRNA target sequence, 5'-AGGAAGACTATGACCATAA-3'; Qiagen, Germany, (Shanghai agent in China)). The BGC-823 cells (n = 2 × 10^5) or a control siRNA (Qiagen) were transfected with siRNA targeted against β2-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 cell lines BGC-823 and SGC-7901 exposed to NE (10 μM; this concentration was chosen based on our previous study). 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...
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 $\beta_2$-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
substantially increased ($P < 0.05$). We also presented evidence that the β₂-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, NE can increase cancer cell invasion through the β₂-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 β₂-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 β₂-AR antagonist ICI 118551 can also reverse NE-induced HIF-1α and Snail protein expressions. These results indicated that β₂-AR–HIF-1α–Snail has a critical role in EMT.

β₂-Adrenergic receptor is required to induce EMT phenomenon for NE. To further evaluate whether β₂-AR activation induced by NE is essential for EMT to occur in gastric adenocarcinoma cells, the effects of β₂-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 β₂-AR. The efficacy of β₂-AR siRNA to knockdown β₂-AR mRNA and protein was confirmed by RT-PCR and Western blot, respectively. We observed that both β₂-AR mRNA and protein levels (Fig. 6a,b) were barely detectable in β₂-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 β₂-AR activity in NE on the EMT of cancer cells.

Hypoxia-inducible factor-1α linked β₂-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...
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.

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.

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.
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 β2-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
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

\[\text{Fig. 6.} \ \beta_2-\text{Adrenergic receptor (β2-AR) siRNA could inhibit norepinephrine (NE)-induced epithelial–mesenchymal transition in gastric adenocarcinoma cells. (a) Efficacy of β2-AR siRNA for knockdown of β2-AR mRNA and protein was confirmed by RT-PCR and Western blot. (b) Quantification of β2-AR mRNA and protein. (c) NE induced cell morphological changes of epithelial–mesenchymal transition in SGC-7901 and BGC-823 cells after β2-AR siRNA. (d) E-cadherin and vimentin fluorescent imaging obtained by a confocal laser scanning microscope showed no disparity between groups. (e) BGC-823 was treated with NE (10 \mu M) with or without β2-AR siRNA. After 48 h, hypoxia-inducible factor-1α (HIF-1α), Snail, E-cadherin, and vimentin proteins were detected by Western blot analysis. (f) Quantification of HIF-1α, Snail, E-cadherin, and vimentin proteins. *P < 0.05 was considered statistically significant. Con, control.}\]
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. 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.
Taken together, the results of this pioneering study imply that the catecholamine hormone NE induces EMT in gastric adenocarcinoma through the regulation of β2-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

We thank the staff of the Biology and Genetics Laboratory at Xi’an Jiaotong University for their technical assistance in this study. The study was funded by the Scientific Grant of Shaanxi-ky201135.

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. *Pancreatology* 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. *Psychiatr Unit* 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, Marielli 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, Sepheton SE. Psychoneuroimmunity 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: 884–8.
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 HX. EMT: a new vision of hypoxia promoting cancer progression. *Cancer Biol Ther* 2011; 11: 714–23.
16. Ksiazkiewicz M, Markiewicz A, Zaczeck 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. *Sem 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, Dania 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 polyposis 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.