Taurine Attenuates Epithelial-Mesenchymal Transition-Related Genes in Human Prostate Cancer Cells

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Abstract Prostate cancer is the most common non-cutaneous cancers among men and the second leading cause of cancer-related deaths among men. Aberrant activation of the epithelial to mesenchymal transition (EMT) has been exhibited to be one of the most common causes of treatment failure and death in cancer patients. In cancer cells with metastatic competence, the E-cadherin switch is a well-established hallmark. Suppression of E-cadherin through its transcriptional repressor SNAIL is thus a determining factor for EMT. TWIST1 is an important transcription factor in EMT, which is present under both physiologic (embryogenesis) and pathologic (metastasis) conditions, and enhances the invasiveness and migration ability of cells. In this study, we investigated the inhibitory effects of taurine on EMT-related

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genes, such as E-cadherin, N-cadherin, TWIST1, ZEB1, SNAIL, and vimentin. EMT markers were detected by RT-PCR and western blotting. The results showed that taurine down-regulated the expression of N-cadherin, TWIST1, ZEB1, SNAIL, and vimentin. In contrast, taurine increased E-cadherin expression. Our findings indicate that taurine has EMT inhibitory effects on human prostate cancer cells.

Keywords Taurine • Prostate cancer • Epitherial to mesenchymal transition

Abbreviations

EMT	Epithelial to mesenchymal transition
PSA	Prostate-specific antigen
SNAIL	Snail family zinc finger 1
TWIST1	Basic helix-loop-helix protein 38
ZEB1	Zinc finger E-box binding homeobox 1

1 Introduction

Taurine, also known as 2-amino ethane sulfonic acid, is synthesized endogenously from cysteine and methionine in the presence of vitamin B6 and is supplied by the diet, particularly from fish and meat. Taurine is present in most organs, showing the highest abundance in the heart and brain. Numerous studies have reported that taurine can function as an anticancer agent (Zhang et al. 2014; El-Houseini et al. 2013).

Prostate cancer is the second leading cause of cancer-related mortality and is one of the most common cancers in men (Ferlay et al. 2013). The incidence of this cancer has recently increased, after which the mortality of prostate cancer also increased (Tormey 2014). In Asia, the incidence of prostate cancer has increased more rapidly than in other developed countries (Tang et al. 2015). We previously showed that taurine inhibited prostate-specific antigen (PSA) levels and migration in human prostate cancer cells (Tang et al. 2015).

In prostate cancer, epithelial-mesenchymal transition (EMT) plays a key role in prostate cancer metastasis (Fu et al. 2016). EMT first occurs during embryogenesis and has been investigated to determine its effects on tumor formation and metastatic growth (Garg 2013). It is a naturally occurring process that drives the transformation of adhesive and non-mobile epithelial-like cells into mobile cells with a mesenchymal phenotype that have the capacity to move to anatomically distant locations (Kalluri and Weinberg 2009). EMT is orchestrated and triggered by various EMT-activating transcription factors, including the basic helix-loop-helix factors (Twist1 and Twist2); zinc-finger E-box-binding homeobox (ZEB)1; the SNAIL family of zinc-finger transcription factors: SNAIL1 (SNAIL), SNAIL2 (SLUG), and SNAIL3 (SMUC); vimentin; and N-cadherin (Perez-Moreno et al. 2001; Nieto 2002; Yang

et al. 2004; Rodriguez et al. 2008). In addition, one of the hallmarks of EMT is the functional loss of E-cadherin, which is thought to be a metastatic suppressor of tumor progression (Hanahan and Weinberg 2011). SNAIL is a prominent inducer of EMT and strongly represses E-cadherin expression (Barrallo-Gimeno and Nieto 2005).

Several studies have reported that the progression of EMT in cancer can be retarded by using plants and chemical substances (Ge et al. 2016; Shankar et al. 2011; Scarpa and Ninfali 2015). However, little is known about the inhibition of EMT in prostate cancer cells by taurine.

Therefore, in the present study, we investigated the effects of taurine on EMT in prostate cancer cells.

2 Methods

2.1 Materials

LNCaPs, which are androgen-dependent human prostate cancer cells, were obtained from the Korean Cell Line Bank (Seoul, South Korea; KCLB numbers: 21740). Taurine and dihydrotestosterone (DHT) were purchased from Sigma-Aldrich. (St. Louis, MO, USA). TRIzol reagent for RNA extraction was obtained from Invitrogen (Carlsbad, CA, USA). Antibodies for primary antibodies and peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other reagents were of the highest grade commercially available at the time of the study.

2.2 Cell Culture

The human prostate cancer cell line, LNCaP, was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a 5% CO_2 atmosphere at 37 °C. The cells were seeded at a density of 3.5×10^5 cells per well in a 13-cm culture dish. After 24 h, the cells were treated with 250, 500, and 1000 μM taurine in the medium. Cells were treated with taurine for 24 h and then harvested.

2.3 Analysis of mRNA Expression

For reverse transcription-polymerase chain reaction (RT-PCR), total cellular RNA was isolated from the cells of each treatment group using TRIzol according to the manufacturer's protocol (Kim et al. 2014). First-strand complementary DNA was

synthesized using Superscript II reverse transcriptase (Invitrogen). The conditions for RT-PCR were the same as those described in a previous study (Kim et al. 2014). The mixture was first incubated for 15 min at 95 °C for initial denaturation, followed by 40 cycles of the amplification step. The following protocol was followed: denaturation for 30 s at 95 °C, annealing at a transitional temperature range from 58 to 62 °C with an increase of 0.5 °C per cycle; and then extension for 30 s at 72 °C. The expression levels of the analyzed genes were normalized to that of GAPDH for each sample and presented as relative mRNA levels.

2.4 Western Blotting

After the indicated treatments, the cells were harvested in PBS and lysed in RIPA buffer (150 mM NaCl, 0.5% deoxycholate, 0.1% nonidet P-40, 0.1% SDS, and 50 mM Tris) containing protease inhibitors (50 mg/mL phenylmethylsulfonyl fluoride, 10 mg/mL aprotinin, 5 mg/mL leupeptin, 0.1 mg/mL NaF, 1 mM DTT, 0.1 mM sodium orthovanadate, and 0.1 mM β-glycerophosphate). Total cellular proteins were quantified using the Bradford procedure and equal amounts of proteins were mixed with loading buffer (25% glycerol, 0.075% SDS, 1.25 mL of 14.4 M 2-mercaptoethanol, 10% bromophenol blue, and 3.13% stacking gel buffer) and fractionated by gel electrophoresis on gradient gels (Novex, CA, USA). Rainbow marker (Novex, Thermo Fisher, Waltham, MA, USA) was used as the molecular weight standard. Proteins were transferred to nitrocellulose membranes (Novex) and blocked for 1.5 h with clear milk (Thermo Scientific). Blots were subsequently incubated with primary antibodies in 1X TBST for 1.5 h. Goat anti-rabbit or goat anti-mouse horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used at a 1:5000 dilution in 1X TBST. Blots were treated with Western Lightning Western Blot Chemiluminescence Reagent (Advansta, Menlo Park, CA, USA) and the proteins were detected by autoradiography (Fujifilm, Tokyo, Japan). β-Actin was used as a loading control.

2.5 Statistical Analysis

All data are presented as the mean ± SE from at least three experiments. Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA). Treatment effects were analyzed using one-way analysis of variance, followed by Dunnett's multiple range tests. For the results, $p < 0.05$ was considered to indicate significance.

3 Results

3.1 Effect of Taurine on the Expression of EMT-Related Genes

In a previous study, we reported that taurine significantly stimulated prostate cancer cell death in a dose-dependent manner at concentrations of 125–1000 μM (Tang et al. 2015). Based on these results, we investigated the effects of taurine (0, 250, 500, and 1000 μM) on EMT in prostate cancer cells. DHT-stimulated human prostate cancer cells, LNCaPs, were treated with media supplemented with taurine for 24 h. After 24 h, the cells were harvested, and total RNA and protein were isolated. The gene expression and protein levels of EMT-related genes including E-cadherin, N-cadherin, TIWST1, ZEB1, SNAIL, and vimentin were measured by RT-PCR and western blotting, respectively. Treatment of LNCaP cells with taurine significantly increased the mRNA expression of the epithelial gene E-cadherin (Fig. 1a and b). In addition, mesenchymal genes were estimated. As shown in Fig. 1a and b, taurine

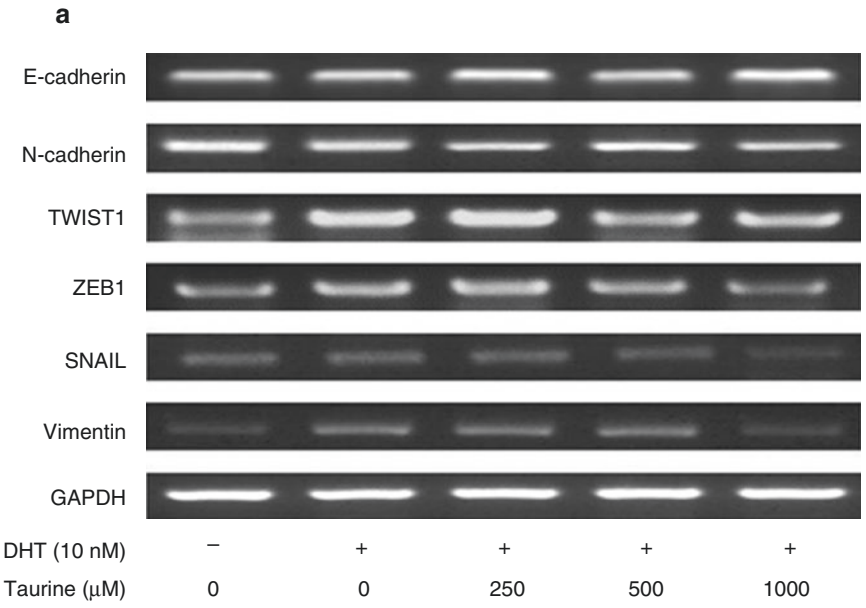

Fig. 1. Effect of taurine on EMT-related gene mRNA levels. LNCaP cells were incubated with or without DHT (10 nM) or taurine (250, 500, and 1000 μM). (**a**) After 24 h, cells were harvested and total RNA was isolated. Gene expression levels of EMT-related genes (E-cadherin, N-cadherin, TWIST1, ZEB1, SNAIL, and vimentin) were measured by RT-PCR. (**b**) The mRNA levels of the analyzed genes were normalized to that of GAPDH for each sample. Means with different letters (a–c) within each graph were significantly different from other samples at $p < 0.05$. CON, control; DHT, dihydrotestosterone

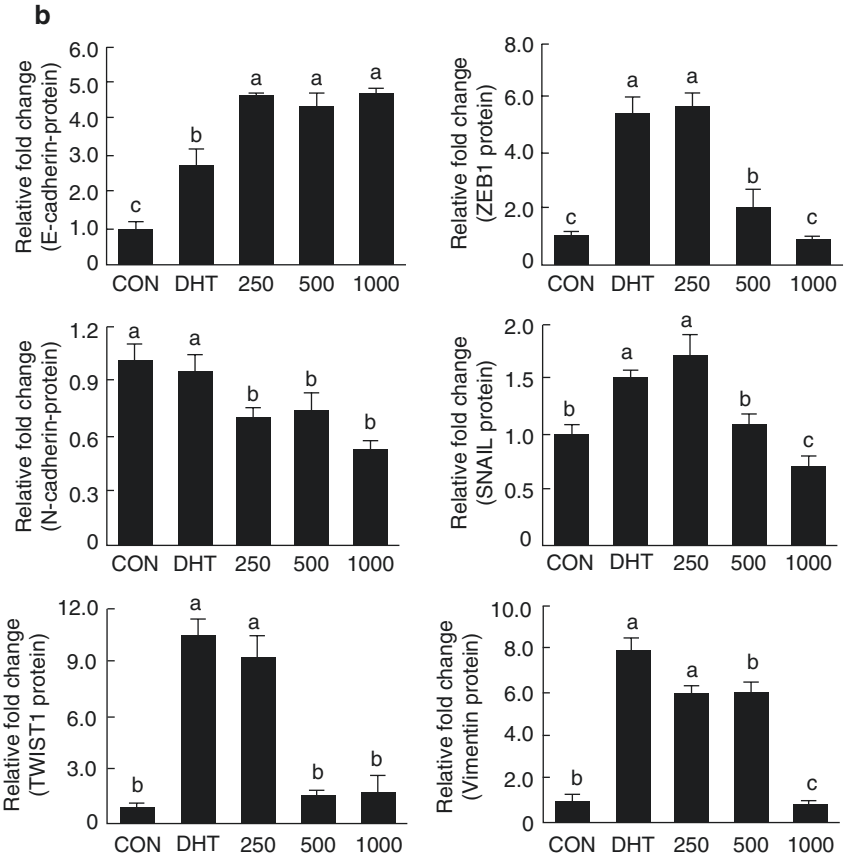

Fig. 1. (continued)

significantly suppressed the mRNA expression of EMT-related genes such as N-cadherin, TIWST1, ZEB1, SNAIL, and vimentin in LNCaP cells. These data suggest that taurine suppresses the mesenchymal condition by regulating the mRNA levels of E-cadherin, N-cadherin, TIWST1, ZEB1, SNAIL, and vimentin in the androgen-dependent human prostate cancer cells LNCaPs.

In addition, we examined the effect of taurine on the protein expression of EMT-related genes. As shown in Fig. 2a and b, taurine significantly suppressed the protein expression of EMT-related genes such as N-cadherin, TIWST1, ZEB1, SNAIL, and vimentin in LNCaP cells. These data suggest that taurine suppresses the mesenchymal condition by regulating the protein and mRNA levels of E-cadherin, N-cadherin, TIWST1, ZEB1, SNAIL, and vimentin in LNCaPs.

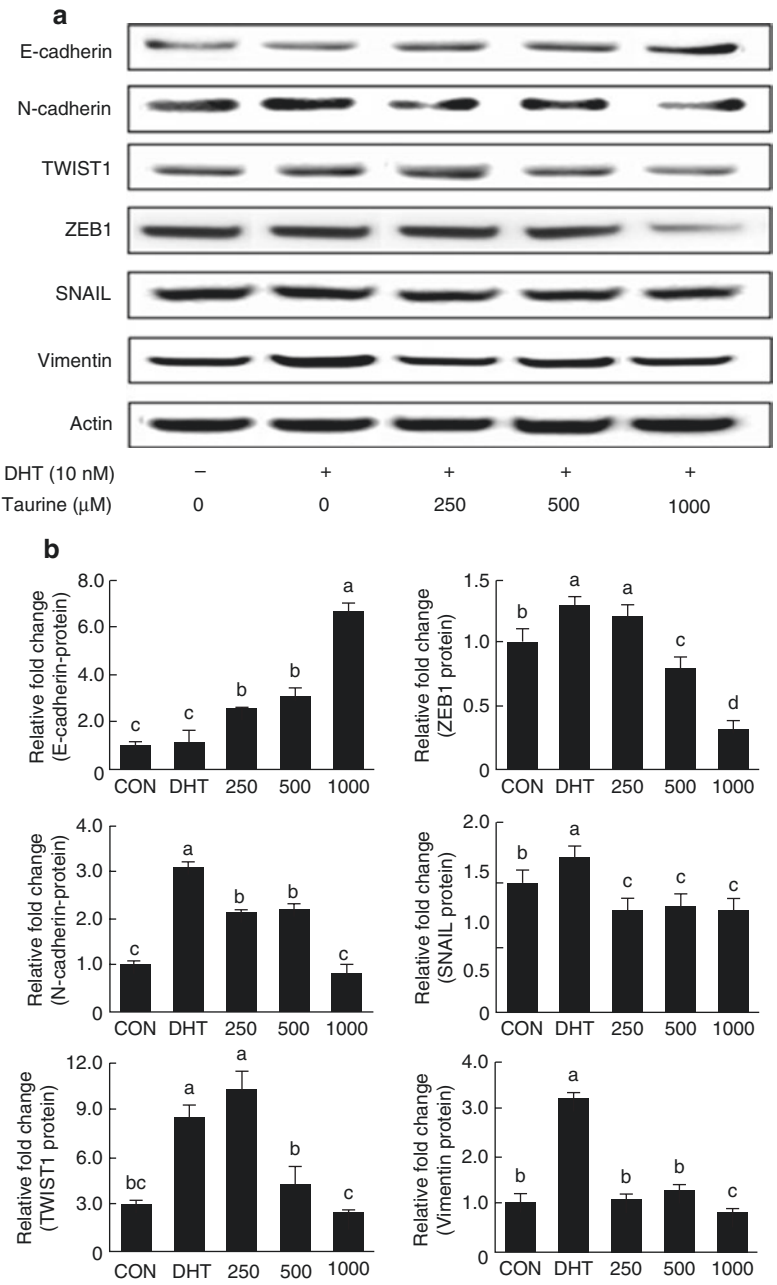

Fig. 2. Effect of taurine on EMT-related gene protein levels. Taurine LNCaP cells were incubated with or without DHT (10 nM) and taurine (250, 500, and 1000 μM). (**a**) After 24 h, the cells were harvested and total protein was isolated. Gene expression levels of EMT-related genes (E-cadherin, N-cadherin, TIWST1, ZEB1, SNAIL, and vimentin) were measured by western blotting. (**b**) The protein levels of the analyzed genes were normalized to that of β-actin for each sample. Means with different letters (a–d) within a graph are significantly different at $p < 0.05$. CON, control; DHT, dihydrotestosterone

4 Discussion

EMT is naturally occurring phenomenon and plays a key role during embryogenesis as well as adult tissue repair and maintenance (Garg 2013). It is represented by specific gene expression pattern changes and the loss of adherent tight junctions that maintain contact of epithelial cell with their neighbors. These factors are related to epithelial genes including E-cadherin, gain of mesenchymal phenotype, including fibro-blastoid morphology, and increased mobility potential to distant sites, which involves mesenchymal genes such as N-cadherin, TIWST1, ZEB1, SNAIL, and vimentin (Fig. 3). Recent increasing evidence has demonstrated the vital process of EMT-activating transcription factors in oncogenic transformation.

EMT is characterized by the downregulation of epithelial markers/tight junction components, desmosomes, cytokeratins, and gain of mesenchymal markers such as reorganization of the cytoskeleton (e.g., switch from cytokeratins to vimentin), and the synthesis of extracellular matrix components and metalloproteases (Thiery et al. 2009). Loss-of-function mutations and promoter hyper-methylation may downregulate E-cadherin expression and function in a number of carcinomas, but modulation of EMT during embryogenesis and cancer progression primarily involves EMT-activating transcription factors (Scheel et al. 2011). In our previous study, we demonstrated that taurine inhibited metalloproteases in human prostate cancer cells (Tang et al. 2015). Moreover, we demonstrated that taurine stimulated the expression of an epithelial markers/tight junction component, E-cadherin. In addition to a mesenchymal switch, these factors control the entire EMT program and endow cancer cells with stem-like characteristics. These migrating cancer stem cells are not only important in the genesis of primary tumors, but also enhance metastasis and possibly the root cause of tumoral chemoresistance and recurrence (Scheel et al. 2011). We also found that taurine suppressed the migration of human prostate cancer cells (Tang et al. 2015).

Binding of TWIST1 to other transcriptional regulators, post-translational modifications, and choice of partner for dimerization regulate the expression of target genes. Classical EMT-inducing pathways, such as TGFβ, Wnt, hypoxia, and ligand

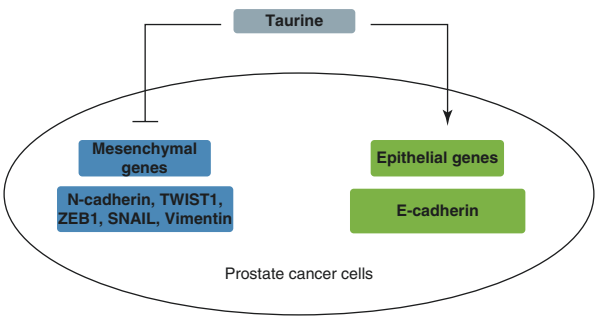

Fig. 3. Schematic illustration of taurine-mediating signaling pathways during epithelial-to-mesenchymal transition in prostate cancer cells

binding activation of receptor tyrosine kinases and inflammatory cytokine receptors, activate TWIST1 and have significant implications in tumor invasion and angiogenesis (Qin et al. 2012; Yang et al. 2008). In addition, the binding of TWIST1 to the H4K20 methyltransferase SET8 represses E-cadherin and activates N-cadherin (Fu et al. 2011; Yang et al. 2012). In this study, taurine inhibited N-cadherin and TWIST1 expression in DHT-stimulated human prostate cancer cells.

The ZEB family consists of the zinc finger/homeodo-main proteins-ZEB1 and ZEB2, which are well conserved among species. These proteins interact with other transcriptional factors and their expression is modulated by post-translational modifications such as SUMOylation by Pc2 or acetylation by p300/pCAF and phosphorylation. These proteins trigger EMT by inhibiting epithelial markers and activating mesenchymal properties (Garg 2013). Additionally, SNAIL-mediated histone modifications are induced to repress E-cadherin. Signals, including TGFβ, Notch, tumor necrosis factor-α, EGF, FGF, Wnt, Shh, and estrogens, regulate SNAIL proteins, not only during development but also in cancer cells (Thiery et al. 2009). In the present investigation, taurine suppressed the levels of ZEB1 and SNAIL as well as vimentin, which is a mesenchymal marker.

5 Conclusion

Our results demonstrated that taurine inhibited human prostate cancer cell metastasis. Our findings also indicate that taurine possesses EMT inhibitory effects in the androgen-dependent human prostate cancer cells, LNCaPs. These *in vitro* results provide a foundation for future studies of this novel therapeutic regimen and suggest that combined use of taurine with other agents can be used to prevent and treat prostate cancer in humans.

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