

P300 promotes migration, invasion and epithelial-mesenchymal transition in a nasopharyngeal carcinoma cell line

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Abstract. A previous study demonstrated that p300 is over-expressed in nasopharyngeal carcinoma (NPC), and that its expression is an independent prognostic factor. The aim of the present study is to investigate the role of *p300* in human NPC development. A small hairpin (sh) RNA lentiviral expression vector targeting the *p300* gene was constructed to suppress the expression of p300 in NPC cells. Knockdown of *p300* was verified by reverse transcription-quantitative polymerase chain reaction and western blotting. Wound-healing, invasion, immunofluorescence and immunoprecipitation assays were performed to assess the influence of p300 on nasopharyngeal tumorigenesis and metastasis *in vitro*. The expression of p300 was upregulated in NPC cell lines. After knockdown of *p300*, the migration and invasion ability of shp300 cells were significantly inhibited ($P < 0.05$). Furthermore, the depletion of p300 expression in NPC cell lines resulted in the upregulation of epithelial phenotype marker E-cadherin and α -catenin, and downregulation of mesenchymal phenotype markers N-cadherin and vimentin. p300 promotes epithelial-mesenchymal transition (EMT) through the acetylation of Smad2 and Smad3 in the tumor growth factor- β signaling pathway. In conclusion, p300 may be involved in the invasion and metastasis of NPC through the induction of EMT.

Introduction

Nasopharyngeal carcinoma (NPC) is a distinct type of cancer of the head and neck which is highly prevalent in Southern

China (1). Radiotherapy (RT) and concurrent chemo-radiotherapy are the primary treatments for NPC in the early and advanced stages, respectively (2). Although early-stage NPC is highly radiocurable, metastasis to regional lymph nodes or distant organs and local recurrence remain major issues for the treatment failure of advanced-stage NPC. The molecular mechanisms underlying NPC development and progression have are not yet completely understood.

p300, a transcriptional co-activator of various transcription factors, has been found to serve a central role in the regulation of gene transcription through its histone acetyltransferase activity. It has been shown to participate in different cellular processes such as DNA damage repair, cell growth, differentiation, apoptosis and migration (3). In a previous study by the authors of the present study (4), it was identified that p300 expression levels were higher in NPC tissues compared with adjacent non-cancerous tissues, and p300 has been shown to be overexpressed in a number of malignancies, such as breast cancer (5), colorectal carcinomas (6), esophageal squamous cell carcinoma (7) and hepatocellular carcinoma (8). In addition, a previous study confirmed that elevated p300 activity is correlated with poor prognosis in NPC (4). These findings suggest that p300 could be considered a rational therapeutic strategy for NPC. Therefore, more extensive studies are required to elucidate the molecular status of the *p300* gene and its potential oncogenic role in NPC.

Epithelial-mesenchymal transition (EMT) has been demonstrated to be a fundamental process that could serve a vital role in tumor progression (9). It is characterized by cells losing their epithelial morphology and acquiring mesenchymal markers. Emerging evidence has suggested that EMT is involved in the metastatic behavior of NPC (10,11). In addition, a previous study demonstrated that p300 may promote cancer progression by inducing EMT in hepatocellular carcinoma (12). However, whether or not p300 may promote cancer progression by inducing EMT in NPC has not been reported.

In the present study, lentivirus-mediated small interfering RNA (siRNA) techniques were applied to produce specific and stable silencing of *p300* in CNE-2 cells. In addition, the study investigated the role of p300 in NPC metastasis and EMT process.

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Materials and methods

Cell culture. Human nasopharyngeal carcinoma cell lines CNE-1, CNE-2, SUNE-5-8F, SUNE-6-10B and control cell line NP69 were grown in Roswell Park Memorial Institute (RPMI)-1640 (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.). All cell lines were cultured in a 5% CO₂ incubator at 37°C. When the cells reached the logarithmic growth phase, succeeding experiments were performed.

Construction of lentivirus vectors. To investigate the function of *p300*, the vector LV-008 (Forevergen Biosciences, Guangzhou, China) with a U6 promoter was used to generate small hairpin (sh)RNA. The *p300* shRNA sequence is as follows: Sense, 5'-AACTGCACAAATGTCTAGTTCTTTTCAAGAGAAAGAAGTAGACATTTGTGCTTTTTTTC-3' and antisense, 3'-TTGACTCTTCTCCCTCAGATATAAAGTTCTCTTTCTTGATCTGTAAACACGAAAAAAGAGCT-5'. Negative Control (NC) were used as the control group: Sense, 5'-AAC TTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCCGAGAATTTTTTTC-3' and antisense, 3'-TTGAAA GAGGCTTGACAGTGCAAAGTTCTTGCCTGTGC AAGCCTCTTAAAAAAGAGCT-5'. Lentiviral vector DNA and package vectors were then transfected into HEK-293T cells using Lipofectamine 2000 (Gibco; Thermo Fisher Scientific, Inc.). Lentivirus supernatants were harvested at 48 and 72 h post-transfection. Lenti-sip300 (shp300) and src-siRNA (NC) were then used to infect CNE-2 cells with 5-10 µg/ml polybrene. After 48 h of infection, the cells were cultured in the medium with 2 µg/ml puromycin for 10-15 days to generate stable cell lines.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) determination. Total RNA was extracted from cultured cells and fresh tissue with TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. RNA was reverse transcribed by M-MLV Reverse Transcriptase (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. Primers for P300 were as follows: Forward, 5'-CGTTGCCCT ATCTCCGTCTC-3' and reverse, 5'-GGGAGCAATCGGGTA ATTTTCC-3'. GAPDH was used as internal control. GAPDH primers were as follows: Forward, 5'-GAGTCAACGGATTTG GTCGT-3' and reverse, 5'-GACAAGCTTCCCCTTCTCAG-3'. qPCR was performed on a MiniOpticon™ Real-Time PCR detection instrument (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions. All reactions were performed in triplicate and the experiments were conducted three times. Relative expression quantifications were performed using the 2^{-ΔΔC_q} method (13).

Western blot. Cells from all experimental groups were collected using a cell scraper and proteins were extracted by cell lysis buffer. Total protein was extracted from human NPC lines and liver tissues using radioimmunoprecipitation assay buffer (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany). Protein concentration was assessed with a Bradford assay. Equal quantities of cell and tissue lysates (30 µg

protein) were separated by 10% SDS-PAGE, transferred onto a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA) and blocked with 5% skimmed milk powder for 1 h at room temperature. Membranes were then incubated with p300 antibody (catalog no. sc-584; 1:250 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) against target proteins at 4°C, followed by washing with TBS and Tween-20 (TBST) overnight. Membranes were then incubated with the horseradish peroxidase-labeled secondary antibody (1:1,000 dilution; YN-16; Forevergen Biosciences) for 1 h at 37°C, and then washed with TBST. The immunoreactive signals were detected with enhanced chemiluminescence (Forevergen Biosciences). GAPDH was used as a loading control.

In vitro cell invasion assay. Invasion assays were performed using transwell chambers (BD Biosciences, Franklin Lakes, NJ, USA). Plates were coated with Matrigel prior to cell seeding, cells were suspended in RPMI-1640 supplemented with 0.1% FBS and added to the upper chambers, and Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) with 10% FBS was added to the bottom chambers. After overnight incubation, non-migratory cells on the upper surface were removed. The cells that had passed through the membrane were analyzed in 6 randomly selected microscopic fields. All experiments were performed in triplicate.

Wound healing assay. Cells were seeded in six-well plates and incubated for 24 h prior to the assay. For the wound healing assay, monolayers were wounded by scraping with the tip of a sterile 200 µl pipette tip. Cell migration to the wounded surface was then monitored by microscopy after 6, 24 and 48 h. Experiments were performed a minimum of three times.

Immunofluorescence. Cells were plated at a density of 2x10⁵ cells on cover glasses (Thermo Fisher Scientific, Inc.) for 24 h, fixed using freshly prepared 4% paraformaldehyde and permeabilized using 0.1% Triton X-100 in TBS. The expression of E-cadherin, α-catenin, vimentin and N-cadherin were detected by incubation at 4°C with primary antibodies against E-cadherin (catalog no. sc-7870; 1:1,000 dilution; Santa Cruz Biotechnology, Inc.), α-catenin (catalog no. BD#610193; 1:1,000 dilution; BD Transduction Laboratories; BD Biosciences), vimentin (catalog no. sc-6260; 1:1,000 dilution; Santa Cruz Biotechnology, Inc.) and N-cadherin (catalog no. sc-53488; 1:1,000 dilution; Santa Cruz Biotechnology, Inc.), and then incubated with Alexa Fluor 488 Donkey anti-Rabbit IgG(H+L) secondary antibody (catalog no. CA21206s; 1:500 dilution; Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 30 min. The coverslips were counterstained with DAPI and imaged with an Axio Imager Z1 Microscope System (Carl Zeiss AG, Oberkochen, Germany).

Immunoprecipitation (IP) assays. For each trial, cell extracts composed of 6.0x10⁶ cells were prepared by solubilization in 400 µl cell lysis buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, proteinase inhibitor cocktail] for 10 min at 4°C. After brief sonication, the lysates were cleared by centrifugation at 15,000 x g for 15 min

at 4°C, and the cell extract was immunoprecipitated with 2 µg smad2/3 (Cell Signaling Technology, Inc., Danvers, MA, USA) and incubated with 30 µl protein A plus Gagarosehydrazide beads (Calbiochem; Merck Millipore), overnight at 4°C. After washing three times with agarose bead washing buffer, immunoprecipitated protein-antibody complexes were subjected to western blotting, as aforementioned, and then detected with Acetylated-Lysine antibody (catalog no. 9441; 1:1,000 dilution; Cell Signaling Technology, Inc.) and Smad2/3 antibody (catalog no. 5678; 1:1,000 dilution; Cell Signaling Technology, Inc.).

Statistical analysis. All the results are expressed as the mean ± standard deviation and analyzed using a Student's t-test between two groups. $P < 0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using SPSS version 13.0 statistical software (SPSS, Inc., Chicago, IL, USA).

Results

Elevated expression of p300 in NPC cell lines. To examine the effects of p300 in functional studies, it is important to select a cell culture system that expresses the appropriate level of endogenous p300. p300 mRNA and protein expression in various human NPC cell lines were confirmed by RT-qPCR and western blot analysis. Western blotting (Fig. 1A) and RT-qPCR (Fig. 1B) results showed enhanced expression of p300 protein and mRNA in all the four NPC cell lines as compared with the immortalized non-tumorigenic cell line NP69. Based on this expression pattern, CNE-2 cells were chosen for the following loss-of-function studies.

Suppression of p300 mRNA and protein expression of cells by lentivirus transduction. To further explore the particular function of p300 in NPC progression, p300 expression was silenced using RNA interference. A lentivirus-delivered vector shp300 and NC was constructed (Fig. 2A). RT-qPCR and western blot were used to confirm the inhibition of p300. As shown in Fig. 2B and C, compared with untransfected cells, treatment with shp300 resulted in a significant reduction of p300 in CNE-2 cells at both protein and mRNA levels ($P < 0.01$), while the p300 protein and mRNA expression was almost unaffected in the NC group. These data suggested that shp300 displays efficient mRNA degradation ability. Thus, shp300 cells were chosen for functional assays.

Suppression of p300 reduced the migration and invasion ability of cells. In order to investigate the potential influence of p300 on cancer cell migration and invasion ability of NPC cells, wound healing and invasion assays were performed. As shown in Fig. 3A, data from the wound healing assay revealed that depletion of p300 in cancer cells strongly reduced cell migration. Similarly, the number of the cells that migrated through the Matrigel significantly decreased in shp300 compared with NC group ($P < 0.01$; Fig. 3B). Taken together, these data indicated that knockdown of p300 decreased the migration and invasion abilities of CNE-2 cells.

p300 promoted EMT in cells. EMT is known to be important for epithelial cancer metastasis (9). In order to study the

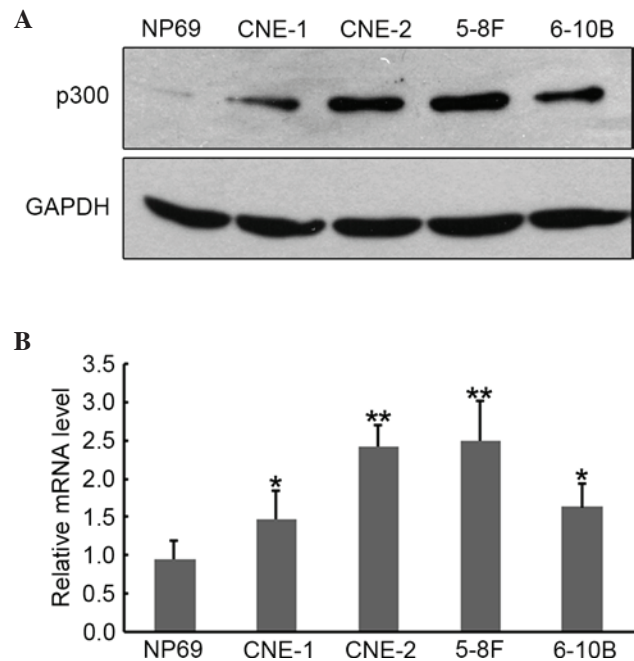


Figure 1. (A) Western blot and (B) reverse transcription-quantitative polymerase chain reaction analysis of p300 expression in nasopharyngeal carcinoma cell lines (CNE-1, CNE-2, 5-8F, 6-10B) and non-cancerous nasopharyngeal cells (NP69). * $P < 0.05$ vs. NP69, ** $P < 0.01$ vs. NP69.

association between p300 and the EMT process, the protein levels of several epithelial markers (E-cadherin, α -catenin) and mesenchymal markers (vimentin, N-cadherin) were detected by western blot analysis. As shown in Fig. 4A, following knockdown of p300 in CNE-2 cells, the protein levels of E-cadherin and α -catenin increased, whereas vimentin and N-cadherin decreased. The EMT phenotype was confirmed by immunofluorescence. As revealed in Fig. 4B, the epithelial markers E-cadherin and α -catenin dramatically increased and the mesenchymal markers vimentin and N-cadherin decreased in shp300 cells compared with the staining observed in the control cells. All of these results suggest that p300 is actively involved in maintaining EMT in NPC cells, and that knockdown of p300 expression markedly increases the expression levels of epithelial markers (E-cadherin, α -catenin) and reduce the expression levels of mesenchymal markers (vimentin, N-cadherin).

p300 promoted EMT through acetylation of Smad2 and Smad3 in the TGF- β signaling pathway. To provide further insights into the signaling mechanisms involved in p300-mediated EMT process, the protein expression levels involved in the TGF- β signaling pathway were measured and it was found that total smad2/3 expression levels remained unchanged when p300 was decreased. An earlier study suggested that both Smad2 and Smad3 directly interact with p300 in the nucleus (14), therefore the present study determined if p300 serves a role in the acetylation of Smad2 and Smad3. These proteins were immunoprecipitated with anti-Smad2 and anti-Smad3 antibodies from NC and shp300 cell lysates, and their acetylation status were determined by anti-acetylated-lysine antibodies. It was observed that knockdown of the p300 gene decreased the acetylation of Smad2 and Smad3 (Fig. 5).

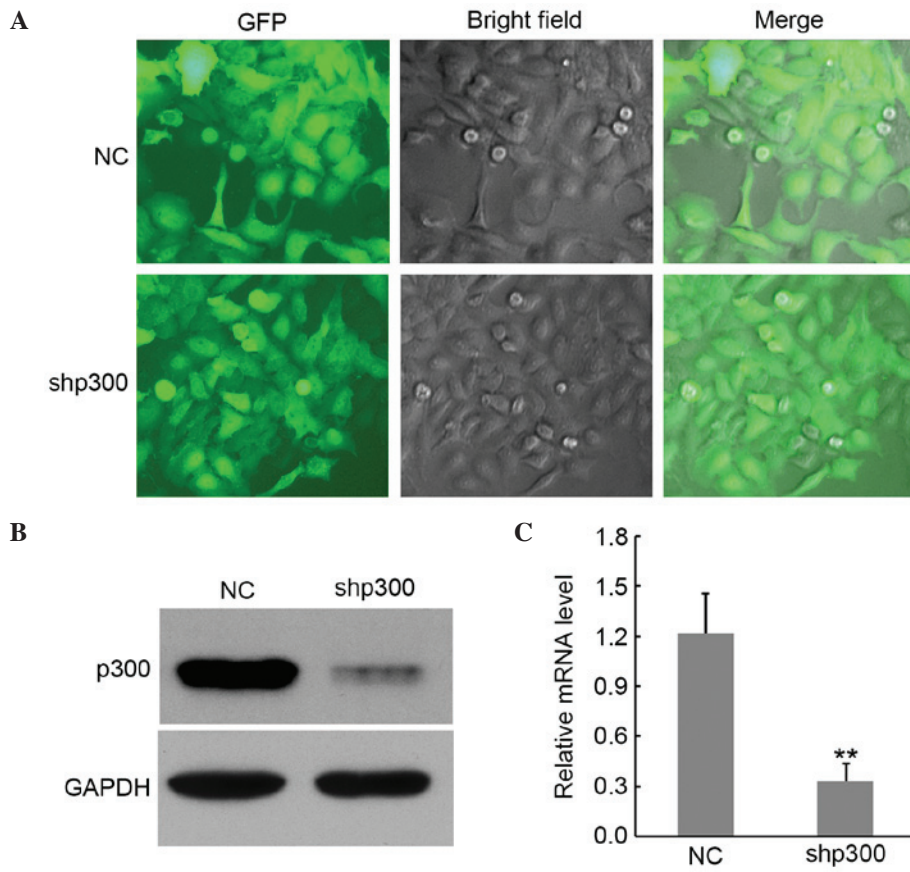


Figure 2. RNA interference in the knockdown p300 gene expression in nasopharyngeal cells. (A) CNE-2 cells were transfected with p300-special and control shRNA. (B) Western blot and (C) reverse transcription-quantitative polymerase chain reaction analysis were used to determine interference efficiency following transfection in CNE-2 cells. These experiments were performed three times. ** $P < 0.01$ vs. NC. GFP, green fluorescent protein; NC, normal control; shp300, small hairpin p300.

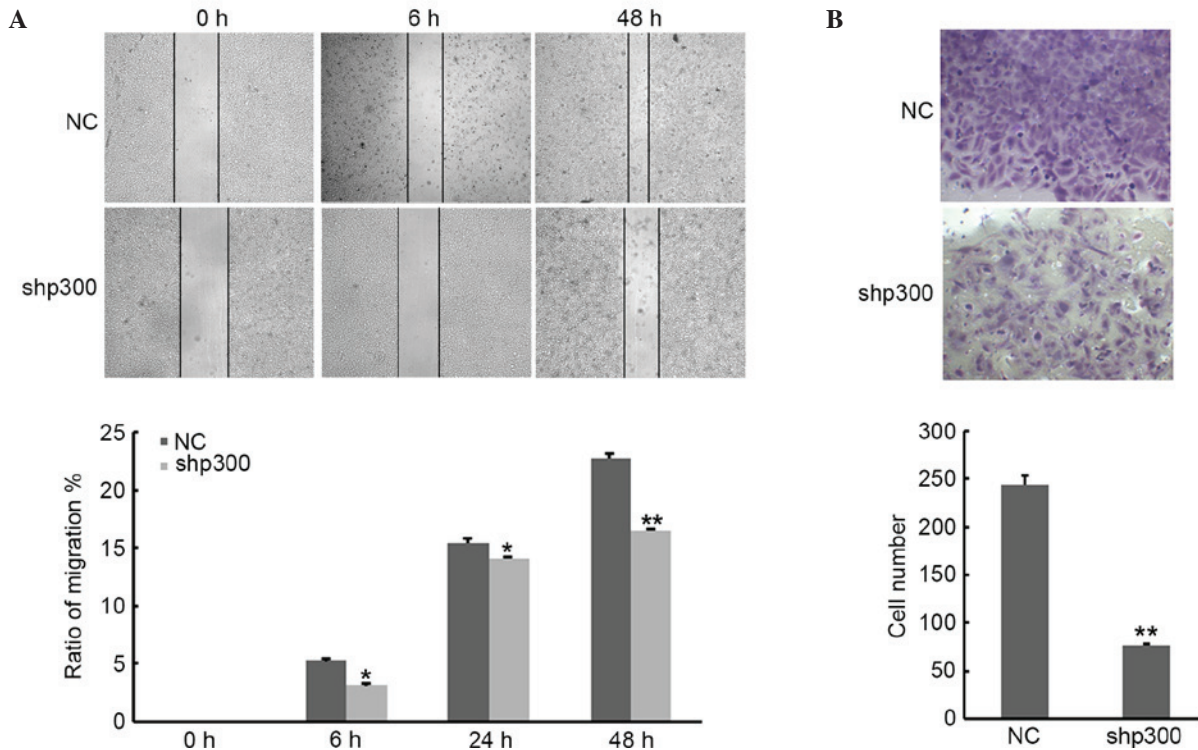


Figure 3. Suppression of p300 affects the invasion and the migration ability of CNE-2 cells. (A) Wound healing assays were performed and quantified in order to evaluate the difference in the migration ability between NC cells and shp300 cells. (B) Cell invasion assay were performed and quantified in NC cells and shp300 cells. * $P < 0.05$ and ** $P < 0.01$ vs. NC. NC, normal control; shp300, small hairpin p300.

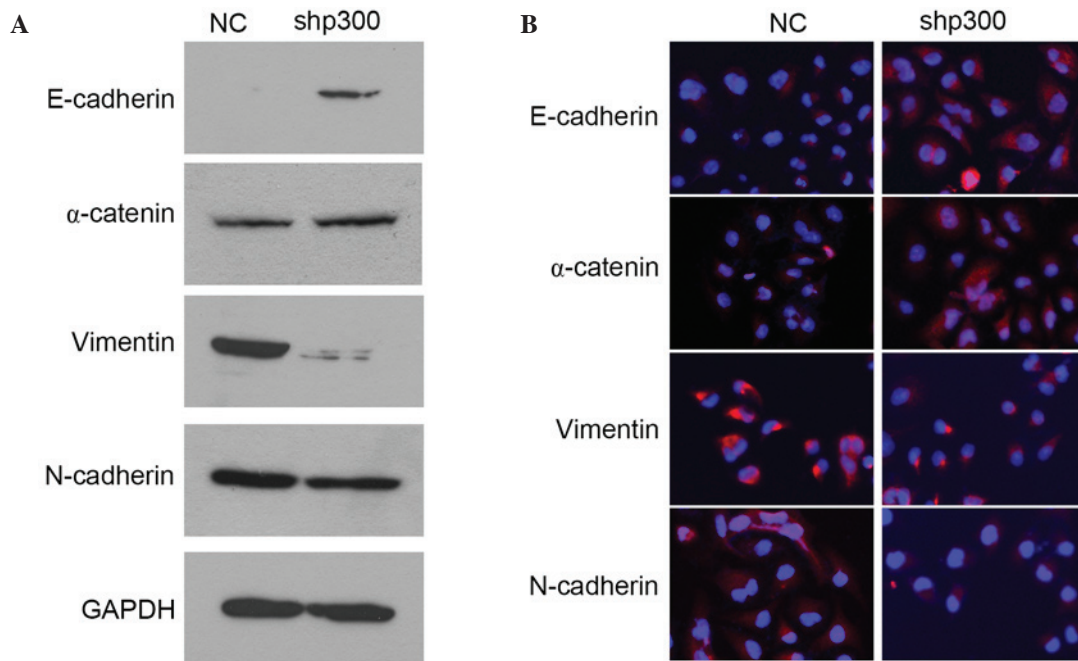


Figure 4. (A) Western blot and (B) immunofluorescence analysis were performed to determine the expression of EMT-associated proteins detection in CNE-2 cells. NC, normal control; shp300, small hairpin p300.

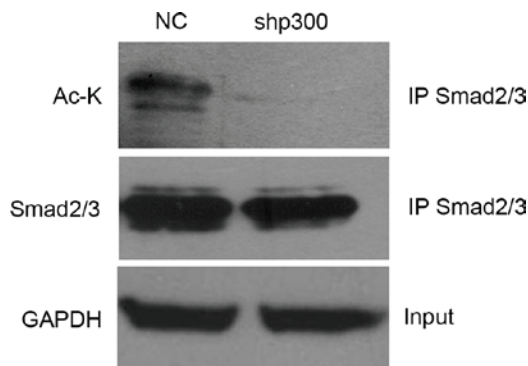


Figure 5. Acetylation level of Smad 2/3 and the expression of Smad2/3 in NC and shp300 cells. NC, normal control; shp300, small hairpin p300; IP, immunoprecipitation.

Discussion

NPC is one of the most common cancers in southern China and Southeast Asia (15,16). Radiotherapy and chemotherapy are the most common treatments of NPC. The 5-year survival rate is excellent for localized stages, but there is no curative treatment for metastatic diseases. Thus it is of paramount interest to identify the molecular mechanisms affecting NPC invasion and metastasis, making it possible to tailor individual treatments.

p300 has been shown to serve a variety of roles in a series of biological processes, such as in cell proliferation, senescence and apoptosis. It functions primarily as a mammalian histone acetyltransferase that acetylates various nuclear proteins. Previously, several studies have demonstrated that p300 was evaluated as a prognostic factor in laryngeal squamous cell carcinoma (17), breast cancer (5), non-small cell

lung cancers (18), small cell lung cancer (19) and esophageal cancers (7).

A previous study demonstrated that the majority of NPCs had a higher expression of p300 compared with in non-cancerous nasopharyngeal mucosal tissues (4). The present study reports, for the first time, that elevated p300 expression in NPC indicates a poor prognosis. In the current study, the expression of p300 at the mRNA and protein levels in NPC cell lines was confirmed by RT-qPCR and western blot analysis, respectively. Consistent with our previous study (4), elevated expression of p300 was observed in invasive NPC cells. These findings suggest that overexpression of p300 may serve a key role in NPC progression. Therefore, the functions of p300 need to be investigated further.

RNA interference-mediated gene silencing is widely used as a powerful tool for the functional analysis of genes. However, the use of RNA interference has been limited by the transient duration of the silencing effect which cannot be passed to cell progeny. To overcome these limitations, a lentiviral vector mediating RNA interference was designed and constructed to obtain a stable *p300* knockdown effect. The results demonstrated that lentivirus-delivered RNA interference could effectively reduce p300 expression.

Both cell migration and invasion are major features that contribute to cancerous progression. A recent study provided evidence that p300 is important for tumor cell growth and migration (20). In the present study, it was observed that knockdown of *p300* significantly inhibited the migration and invasiveness of CNE-2 cells. These findings are in accordance with a previous report which suggested that inhibition of p300 reduced migration and invasion in cultured LM3 cells (21). However, the exact biological mechanisms underlying p300-mediated migration and invasion inhibition during NPC development are unknown.

EMT is considered to be a critical early event involved in the invasion and metastasis of NPC. During EMT, epithelial cells lose their characteristic cell-cell adhesion structures and cell polarity, reduce levels of the epithelial cell surface marker E-cadherin and increase levels of the mesenchymal markers N-cadherin and vimentin. However, the role of p300 in this process remains unclear. It has been reported that the knockdown of p300 expression in hepatoma cells recovered E-cadherin expression, inhibited the translocation of β -catenin into the nuclei and contributes towards the promotion of cell proliferation during tumor pathogenesis (12). Peña *et al* (22) show that p300 levels are important factors in the control of EMT-related molecules, such as ZEB1 and SNAIL in human colon cancer. In the present study, the expression levels of epithelial markers (E-cadherin, α -catenin) were increased when p300 was downregulated, whereas expression of mesenchymal markers (vimentin, N-cadherin) were decreased.

Previously, several studies have suggested that TGF- β signaling serves a role in the control of the invasiveness of NPC cells (23,24). In addition, TGF- β signaling is known to serve a central role in the regulation of EMT (25). The direct downstream target of activated TGF- β is the Smad family. Ko *et al* (14) identified that the acetylation of Smad2 and Smad3 by p300/CBP are essential for the regulation of TGF- β -induced transcriptional activation of EMT markers in human lung cancer cells. Furthermore, it was reported that acetylation of Smad2/3 by p300/CBP is essential for the activation of the transcription factor promoter complex in the TGF- β signaling pathway (26). The data from the present study demonstrated that p300 knockdown resulted in down-regulation of the acetylation of smad2/3. However, a change in Smad2/3 expression was not observed. Taken together, the present study shows that p300 serves an important role in regulating NPC development and metastasis by inducing EMT via acetylation of Smad2 and Smad3 in the TGF- β signaling pathway. Therefore, p300 is considered a novel target for the prevention of EMT. These findings highlight the possibilities that p300 could be a potential target for the therapeutic intervention of NPC.

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References

1. Yu MC and Yuan JM: Epidemiology of nasopharyngeal carcinoma. *Semin Cancer Biol* 12: 421-429, 2002.
2. Zhang L, Zhao C, Ghimire B, Hong MH, Liu Q, Zhang Y, Guo Y, Huang YJ and Guan ZZ: The role of concurrent chemoradiotherapy in the treatment of locoregionally advanced nasopharyngeal carcinoma among endemic population: A meta-analysis of the phase III randomized trials. *BMC Cancer* 10: 558, 2010.
3. Goodman RH and Smolik S: CBP/p300 in cell growth, transformation, and development. *Genes Dev* 14: 1553-1577, 2000.
4. Liao ZW, Zhou TC, Tan XJ, Song XL, Liu Y, Shi XY, Huang WJ, Du LL, Tu BJ and Lin XD: High expression of p300 is linked to aggressive features and poor prognosis of nasopharyngeal carcinoma. *J Transl Med* 10: 110, 2012.
5. Xiao XS, Cai MY, Chen JW, Guan XY, Kung HF, Zeng YX and Xie D: High Expression of p300 in human breast cancer correlates with tumor recurrence and predicts adverse prognosis. *Chin J Cancer Res* 23: 201-207, 2011.
6. Ishihama K, Yamakawa M, Semba S, Takeda H, Kawata S, Kimura S and Kimura W: Expression of HDAC1 and CBP/p300 in human colorectal carcinomas. *J Clin Pathol* 60: 1205-1210, 2007.
7. Li Y, Yang HX, Luo RZ, Zhang Y, Li M, Wang X and Jia WH: High expression of p300 has an unfavorable impact on survival in resectable esophageal squamous cell carcinoma. *Ann Thorac Surg* 91: 1531-1538, 2011.
8. Li M, Luo R, Chen JM, Cao Y, Lu JB, He JH, Wu QL and Cai MY: High expression of transcriptional coactivator p300 correlates with aggressive features and poor prognosis of hepatocellular carcinoma. *J Transl Med* 9: 5, 2011.
9. Thiery JP, Acloque H, Huang RY and Nieto MA: Epithelial-mesenchymal transitions in development and disease. *Cell* 139: 871-890, 2009.
10. Luo WR, Chen XY, Li SY, Wu AB and Yao KT: Neoplastic spindle cells in nasopharyngeal carcinoma show features of epithelial-mesenchymal transition. *Histopathology* 61: 113-122, 2012.
11. Song LB, Li J, Liao WT, Feng Y, Yu CP, Hu LJ, Kong QL, Xu LH, Zhang X, Liu WL, *et al*: The polycomb group protein Bmi-1 represses the tumor suppressor PTEN and induces epithelial-mesenchymal transition in human nasopharyngeal epithelial cells. *J Clin Invest* 119: 3626-3636, 2009.
12. Yokomizo C, Yamaguchi K, Itoh Y, Nishimura T, Umemura A, Minami M, Yasui K, Mitsuyoshi H, Fujii H, Tochiki N, *et al*: High expression of p300 in HCC predicts shortened overall survival in association with enhanced epithelial mesenchymal transition of HCC cells. *Cancer Lett* 310: 140-147, 2011.
13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25: 402-408, 2001.
14. Ko H, So Y, Jeon H, Jeong MH, Choi HK, Ryu SH, Lee SW, Yoon HG and Choi KC: TGF- β 1-induced epithelial-mesenchymal transition and acetylation of Smad2 and Smad3 are negatively regulated by EGCG in human A549 lung cancer cells. *Cancer Lett* 335: 205-213, 2013.
15. Wei WI and Sham JS: Nasopharyngeal carcinoma. *Lancet* 365: 2041-2054, 2005.
16. Chang ET and Adami HO: The enigmatic epidemiology of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev* 15: 1765-1777, 2006.
17. Chen YF, Luo RZ, Li Y, Cui BK, Song M, Yang AK and Chen WK: High expression levels of COX-2 and P300 are associated with unfavorable survival in laryngeal squamous cell carcinoma. *Eur Arch Otorhinolaryngol* 270: 1009-1017, 2013.
18. Hou X, Li Y, Luo RZ, Fu JH, He JH, Zhang LJ and Yang HX: High expression of the transcriptional co-activator p300 predicts poor survival in resectable non-small cell lung cancers. *Eur J Surg Oncol* 38: 523-530, 2012.
19. Gao Y, Geng J, Hong X, Qi J, Teng Y, Yang Y, Qu D and Chen G: Expression of p300 and CBP is associated with poor prognosis in small cell lung cancer. *Int J Clin Exp Pathol* 7: 760-767, 2014.
20. Zhou J, Zhan S, Tan W, Cheng R, Gong H and Zhu Q: P300 binds to and acetylates MTA2 to promote colorectal cancer cells growth. *Biochem Biophys Res Commun* 444: 387-390, 2014.
21. Fermento ME, Gandini NA, Salomón DG, Ferronato MJ, Vitale CA, Arévalo J, López Romero A, Nuñez M, Jung M, Facchinetti MM and Curino AC: Inhibition of p300 suppresses growth of breast cancer. Role of p300 subcellular localization. *Exp Mol Pathol* 97: 411-424, 2014.
22. Peña C, García JM, García V, Silva J, Domínguez G, Rodríguez R, Maximiano C, García de Herrerros A, Muñoz A and Bonilla F: The expression levels of the transcriptional regulators p300 and CtBP modulate the correlations between SNAIL, ZEB1, E-cadherin and vitamin D receptor in human colon carcinomas. *Int J Cancer* 119: 2098-2104, 2006.

23. Zhao L, Lin L, Pan C, Shi M, Liao Y, Bin J and Liao W: Flotillin-2 promotes nasopharyngeal carcinoma metastasis and is necessary for the epithelial-mesenchymal transition induced by transforming growth factor- β . *Oncotarget* 6: 9781-9793, 2015.
24. Xiao J, Xiang Q, Xiao YC, Su ZJ, Huang ZF, Zhang QH, Tan Y, Li XK and Huang YD: The effect of transforming growth factor-beta1 on nasopharyngeal carcinoma cells: Insensitive to cell growth but functional to TGF-beta/Smad pathway. *J Exp Clin Cancer Res* 29: 35, 2010.
25. Morrison CD, Parvani JG and Schiemann WP: The relevance of the TGF- β paradox to EMT-MET programs. *Cancer Lett* 341: 30-40, 2013.
26. Inoue Y, Itoh Y, Abe K, Okamoto T, Daitoku H, Fukamizu A, Onozaki K and Hayashi H: Smad3 is acetylated by p300/CBP to regulate its transactivation activity. *Oncogene* 26: 500-508, 2007.