MORF4L1 suppresses cell proliferation, migration and invasion by increasing p21 and E-cadherin expression in nasopharyngeal carcinoma

YI SANG[1*], RUHUA ZHANG[2*], LONGHUA SUN[3*], KADDIE KWOK CHEN[4], SI-WEI LI[5], LONGXIN XIONG[1], YONGJIAN PENG[2], LEI ZENG[6] and GUOFU HUANG[1]

[1]Nanchang Key Laboratory of Cancer Pathogenesis and Translational Research, Center Laboratory, The Third Affiliated Hospital, Nanchang University, Nanchang, Jiangxi 330008; [2]State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060; [3]Respiratory Department, Nanchang Hospital of Integrative Traditional Chinese and Western Medicine, Jiangxi University of Traditional Chinese Medicine, Nanchang, Jiangxi 330008, P.R. China; [4]College of Arts and Sciences, Cornell University, Ithaca, NY 14850, USA; [5]Department of Radiation Oncology, The Affiliated Hospital of Guilin Medical University, Guilin, Guangxi 541000; [6]Department of Radiation Oncology, Jiangxi Cancer Hospital, Nanchang, Jiangxi 330029, P.R. China

Received February 13, 2017; Accepted September 14, 2018

DOI: 10.3892/ol.2018.9588

Abstract. Mortality factor 4-like 1 (MORF4L1) is a member of a subgroup of histone acetyltransferases and belongs to the mortality factor on chromosome 4 (MORF4) class of proteins. However, the role of MORF4L1 in cancers is largely unknown. Using reverse transcription-quantitative polymerase chain reaction and published datasets, the present study demonstrated that the expression of MORF4L1 is decreased in several cancers, including nasopharyngeal carcinoma (NPC). Additionally, the methylation rate of the promoter of MORF4L1 was identified to be significantly higher in tumour cells than in normal cells. The ectopic expression of MORF4L1 was also revealed to inhibit cell proliferation, colony formation, migration and invasion in NPC, whereas the knockdown of MORF4L1 promoted cell proliferation, colony formation, migration and invasion. Mechanistically, the present study demonstrated that MORF4L1 functions as a tumour suppressor by increasing p21 and E-cadherin levels. These findings may be useful novel targets for treating patients with NPC.

Correspondence to: Professor Guofu Huang, Nanchang Key Laboratory of Cancer Pathogenesis and Translational Research, Center Laboratory, The Third Affiliated Hospital, Nanchang University, 128 Xiangshan North Road, Nanchang, Jiangxi 330008, P.R. China
E-mail: ncsdyyy222@163.com

Dr Lei Zeng, Department of Radiation Oncology, Jiangxi Cancer Hospital, 519 Beijing East Road, Nanchang, Jiangxi 330029, P.R. China
E-mail: 232404116@qq.com

[*]Contributed equally

Key words: MORF4L1, cell proliferation, migration, invasion, NPC

Introduction

Nasopharyngeal carcinoma (NPC) is highly prevalent in Southern China and Southeast Asia with an incidence rate of 15-50 cases/100,000 people (1-3). NPC has the highest metastasis rate among the various types of head and neck cancer, with 74.5 and 19.9% of patients presenting with regional lymph node and distant metastasis respectively, including liver and lung metastasis at the time of diagnosis (1,4). The dysregulation of oncogenes or tumour suppressor genes in NPC is associated with the initiation and progression of cancer, and resistance to therapy (1,3,5-7). Our group previously identified that a transcription factor Telomere length regulation protein TEL2, and a cell membrane protein carbonic anhydrase IX, served a key role in cell proliferation and metastasis in NPC (1,3). Although some progress, including microRNAs, long non-coding RNAs and kinases serving key roles in NPC progression, has been made recently (8-11), the molecular mechanisms of NPC are poorly understood.

Human mortality factor 4-like 1 (MORF4L1) is a member of a subgroup of histone acetyltransferases and belongs to the mortality factor on chromosome 4 (MORF4) class of proteins (12). MORF4L1 and MORF4 share a 96% homology in their amino acid sequences (13). Unlike the majority of histone acetyltransferases that are known for activating gene transcription and promoting cell proliferation, MORF4 was initially cloned and characterized as a senescence gene; this is because the cellular expression of MORF4 induces cell death and senescence (14-16). MORF4L1 homodimerization is essential for facilitating the formation and functionality of

a complex that represses cell proliferation (16). Additionally, MORF4L1 functions during embryonic development via chromatin remodeling and transcriptional regulation (17), and MORF4L1 mediates epithelial cell death in a mouse model of pneumonia (18). In cancer, MORF4L1 acts as a nuclear ligand for the fungal galectin Agrocybe aegerita lectin and serves a key role in its antitumour activity (19). MORF4L1 stimulates homology-directed repair of chromosomal breaks by interacting with BRCA2 DNA repair associated, partner and localizer of BRCA2, RAD51 recombinase and replication protein A1 (20,21). However, little is known regarding the role of MORF4L1 in NPC.

In the present study, the expression of MORF4L1 in clinical NPC tissues was examined and compared with adjacent, non-cancerous tissues, and also sought to evaluate the effects of MORF4L1 expression on NPC cell phenotypes *in vitro*. The findings suggest that MORF4L1 inhibits cell proliferation and migration by increasing the expression levels of p21 and E-cadherin.

Materials and methods

Cells and reagents. The cell lines NP69, 5-8F, 6-10B and SUNE1 were obtained from the group of Tiebang Kang (Sun Yat-sen University Cancer Center, Guangzhou, China). The cell lines 6-10B, 5-8F and SUNE1 were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and maintained at 37˚C in an incubator containing 5% CO_2. The NP69 cell line was maintained in keratinocyte serum-free medium supplemented with 4 μg/ml human epidermal growth factor and 40 μg/ml bovine pituitary extract (Gibco; Thermo Fisher Scientific, Inc.; catalogue no. 17005-042) and maintained at 37˚C in an incubator containing 5% CO_2. All cell lines used in this study were authenticated through short tandem repeat profiling within 6 months of initiating this project, and the cells were cultured for <2 months.

Plasmids. The full-length cDNA of human MORF4L1 was cloned into the pSin-puro vector (Focus Bioscience Co., Ltd., Nanchang, China). Short hairpin (sh) RNA-NC targeting EGFP (with no known targets in the human genome) was cloned into the pFCL2.0 vector with the following sequence: 5'-GGGCGAGGAGCTGTTCACCG-3'. The oligonucleotide sequence for the human MORF4L1 short hairpin RNA (shRNA)-MORF4L1 was 5'-GTTGCCATAAAGGACAAA CAA-3' (Focus Bioscience Co., Ltd.).

Antibodies. Mouse monoclonal anti-β-actin (catalogue no. FB075) antibody was obtained from Nanchang Focus Bioscience Co., Ltd, and the species specificity is human, mouse, rat and pig. Anti-E-cadherin (catalogue no. 14472) and Anti-p21 (catalogue no. 2947) antibodies were obtained from Cell Signalling Technology, Inc. (Danvers, MA, USA), the species specificity of anti-E-cadherin is human, mouse and rat, and the species specificity of anti-p21 is human and monkey. Anti-MORF4L1 (catalogue no. HPA042360) antibody was obtained from Sigma-Aldrich (Merck KGaA; Darmstadt, Germany), the species specificity of anti-MORF4L1 is human and rat.

Stable cell lines. Subsequently, 3 μg pSin-puro-MORF4L1 (Focus Bioscience Co., Ltd.), 3 μg pSin-puro-empty vector (Focus Bioscience Co., Ltd.), 3 μg shRNA-NC (Focus Bioscience Co., Ltd.) or 3 μg shRNA-MORF4L1 (Focus Bioscience Co., Ltd.) was co-transfected with 3 μg pMD2.G and 3 μg psPAX2 into 293 cells for 48 h. The recombinant viruses were subsequently collected and added to NPC cells, which were cultured with 8 μg/ml polybrene for 24 h at 37˚C. The stable lines were selected by treating the cells with 1 μg/ml puromycin for 2 weeks (Focus Bioscience Co., Ltd.).

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RNA extraction and RT-qPCR procedures were performed as previously described (22,23). Briefly, total RNA obtained from cell lines was isolated using TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. First-strand cDNA was synthesized using the RevertAid™ First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). Subsequently, the qPCR reaction was performed in a CFX96 Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SYBR® Green mix (Tiangen Biotech Co., Ltd., Beijing, China). Thermal cycling of the qPCR reaction was initiated with a denaturation step at 95˚C for 15 min, and consisted of 40 cycles (denaturation at 95˚C 15 sec, annealing at 60˚C for 30 sec and elongation at 72˚C for 30 sec). The amplified products were examined using the $2^{\Delta\Delta Cq}$ method (24), and each sample was calibrated to the expression level of the housekeeping gene GAPDH. The primers employed for amplifying MORF4L1, p21, E-cadherin and GAPDH were validated. The sequences for the primers were as follows: MORF4L1 forward (F), 5'-AGCAATGTT GGCTTATACACCTC-3' and reverse (R), 5'-AGCTTTCCG ATGGTACTCAGG-3'; GAPDH F, 5'-ACAGTCAGCCGC ATCTTCTT-3' and R, 5'-GACAAGCTTCCCGTTCTCAG-3'; E-cadherin F, 5'-AATAGTGCCTAAAGTGCTGC-3' and R, 5'-AGACCCACCTCAATCATCCT-3'; P21 F, 5'-CGATGG AACTTCGACTTTGTCA-3' and R, 5'-GCACAAGGGTAC AAGACAGTG-3'.

Cell proliferation and cell viability assays. *In vitro* cell proliferation and cell viability assays were performed using a Cell Counting Kit-8 (CCK-8) assay. To assess cell proliferation, the cell lines in the present study were seeded in 96-well plates at a density of $1x10^3$ cells/well and incubated for 1, 2, 3, 4 and 5 days. Subsequently, 10 μl of the CCK-8 reagent (catalogue no. C0038; Beyotime Institute of Biotechnology, Haimen, China) was added to each well and incubated for 1.5 h. The absorbance value of each well was measured at 450 nm. For each experimental condition, six wells were evaluated.

Transwell assays. For the Transwell migration assay, $5x10^4$ cells in the present study suspended in 200 μl of serum-free DMEM were added to cell culture inserts, which had 8-μm microporous filters and no extracellular matrix coating (BD Biosciences, Franklin Lakes, NJ, USA). DMEM supplemented 10% FBS was then added to the bottom chamber. Following a 24 h incubation, the cells on the lower surface of the filter were fixed with 10% formalin for 15 min at room temperature, stained with 0.1% crystal violet for 60 min

at room temperature, and examined using a light microscope (magnification, x100). For the *in vitro* invasion assay, a total of $8x10^4$ cells were resuspended in 200 μl of serum-free DMEM and added to the cell culture inserts, which had 8-μm microporous filters and were coated with Matrigel (BD Biosciences). DMEM supplemented with 10% FBS was then added to the bottom chamber and the chamber was incubated for 24 h. The mean number of migrated or invaded cells in three randomly selected optical fields (x100 magnification) from triplicate filters was calculated.

Wound-healing assay. Cell motility was assessed by measuring the movement of NP69, 5-8F, 6-10B and SUNE1 cells from into a scraped cellular area created using a 200-μl pipette tip, and the wound closure was observed at 0, 24 and 48 h. The cells were imaged under a light microscope (magnification, x40).

Western blotting. Western blotting procedures were performed as described previously (1,22). Briefly, cultured cells from all cell lines were lysed in ice-cold radioimmunoprecipitation assay lysis buffer (cat. no., P0013C; Beyotime Institute of Biotechnology) at 4˚C for 30 min. Following centrifugation (12,000 x g) at 4˚C for 20 min, the lysates were obtained and protein concentration was determined with the BCA method. Equal amounts of protein (30 μg/lane) were separated by 10% SDS-PAGE gels; the conditions were: Voltage of 100 V for 2.5 h at room temperature. Proteins were then transferred to polyvinylidene difluoride membranes; the conditions were: Electrical current of 250 mA at 4˚C for 2 h. To block the non-specific binding sites, the membranes were incubated with 5% non-fat milk (in Tris-buffered saline with 0.1% Tween-20) at room temperature for 60 min, and then probed with the following primary antibodies: β-actin (cat. no., FB075; Focus Bioscience, Nanjing, China), E-cadherin (cat. no., 14472; Cell Signalling Technology, Inc.), p21 (cat. no., 2947; Cell Signalling Technology, Inc.), MORF4L1 (cat. no., HPA042360; Sigma-Aldrich; Merck KGaA) at a dilution of 1:1,000 overnight at 4˚C. Subsequently, the membranes were incubated with anti-Rabbit IgG Secondary Antibody Peroxidase Conjugated (cat. no., W401B; Promega Corporation, Madison, WI, USA) and anti-Mouse IgG Secondary Antibody Peroxidase Conjugated (cat. no., W402B; Promega Corporation) with the dilution 1:10,000 at room temperature for 1 h. Specific protein bands were visualized using the BeyoECL Moon detection system (catalogue no. P0018F; Beyotime Institute of Biotechnology) and exposed to radiographic film. Densitometry analysis of the protein bands was performed using ImageJ 1.42 software (National Institutes of Health, Bethesda, MD, USA).

Colony formation assay. Cells used from the aforementioned cell lines in the present study were plated in six-well culture plates at a density of $2.5x10^2$ cells/well. Each group included three wells. The cells were incubated for 15 days at 37˚C, washed twice with PBS and were fixed with 10% formalin at room temperature for 15 min and stained with 0.1% crystal violet at room temperature for 60 min. A cluster containing ≥50 cells was counted as a colony, and was performed with a light microscope (magnification, x100).

RNA interference. Effective small interfering (si)RNA oligonucleotides targeting p21 and E-cadherin with sequences of 5'-CCCUGUUAGUAACGGCAAA-3' and 5'-AUACCA GAACCUCGAACUAUA -3', respectively, were synthesized by Guangzhou RiboBio Co., Ltd., (Guangzhou, China). Approximately $2x10^5$ NPC cells/well were seeded in a six-well tissue culture dish the day prior to transfection. Transfection was performed according to the manufacturer's protocol using Lipofectamine RNAiMAX transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and 50 nM siRNA. At 48 h following transfection, RT-qPCR assays were performed.

Study approval. The use of human NPC tissues was reviewed and approved by the Ethical Committee of Jiangxi Cancer Hospital (Nanchang, China) and was performed in accordance with the approved guidelines. Written informed consent was obtained from all patients. A total of 19 tumor specimens were collected from patients with NPC (median age, 46 years; age range, 29-69 years; male:female ratio, 10:9) with resection between January 2013 and December 2017, and inclusion criteria were as follow: Initial diagnosis of NPC. Exclusion criteria included: Previous radiotherapy, chemotherapy or surgery, with the exception of diagnostic procedures, to the primary tumor or nodes; and previous malignancy or other concomitant malignant disease. A total of 13 normal nasopharyngeal epithelium specimens were collected from healthy participants (median age, 41 years; age range, 32-66 years; male: female ratio, 6:7) with resection between January 2013 and December 2017. All clinical samples were obtained from the Department of Radiation Oncology at the Jiangxi Cancer Hospital (Nanchang, China).

DNA methylation analysis. Methylation data of MORF4L1 were obtained from the MethHC database (http://methhc.mbc.nctu.edu.tw/php/index.php). All the online data was used for analyzing the methylation rate of MORF4L1 promoter with MethHC 1.0.3. software (http://methhc.mbc.nctu.edu.tw/php/index.php).

Statistical analysis. All statistical analyses were performed using SPSS for Windows, version 16.0 (SPSS, Inc., Chicago, IL, USA). All values from the *in vitro* assays are expressed as the mean ± standard deviation or mean ± standard error of the mean of at least three independent experiments or replicates. Paired Student's t-test was used to compare two groups while comparisons between multiple groups were performed by using one-way analysis of variance followed by a Tukey-Kramer post-hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

MORF4L1 expression levels are decreased in various cancer types. MORF4L1 expression levels were significantly lower in NPC tissues compared with normal nasopharyngeal epithelial tissues at the mRNA level as quantified by RT-qPCR (Fig. 1A). Likewise, MORF4L1 mRNA levels were significantly downregulated in NPC cell lines when compared with the normal nasopharyngeal epithelial cell line (NP69) (Fig. 1B). Whether the pattern of MORF4L1 expression in normal vs. tumor tissues

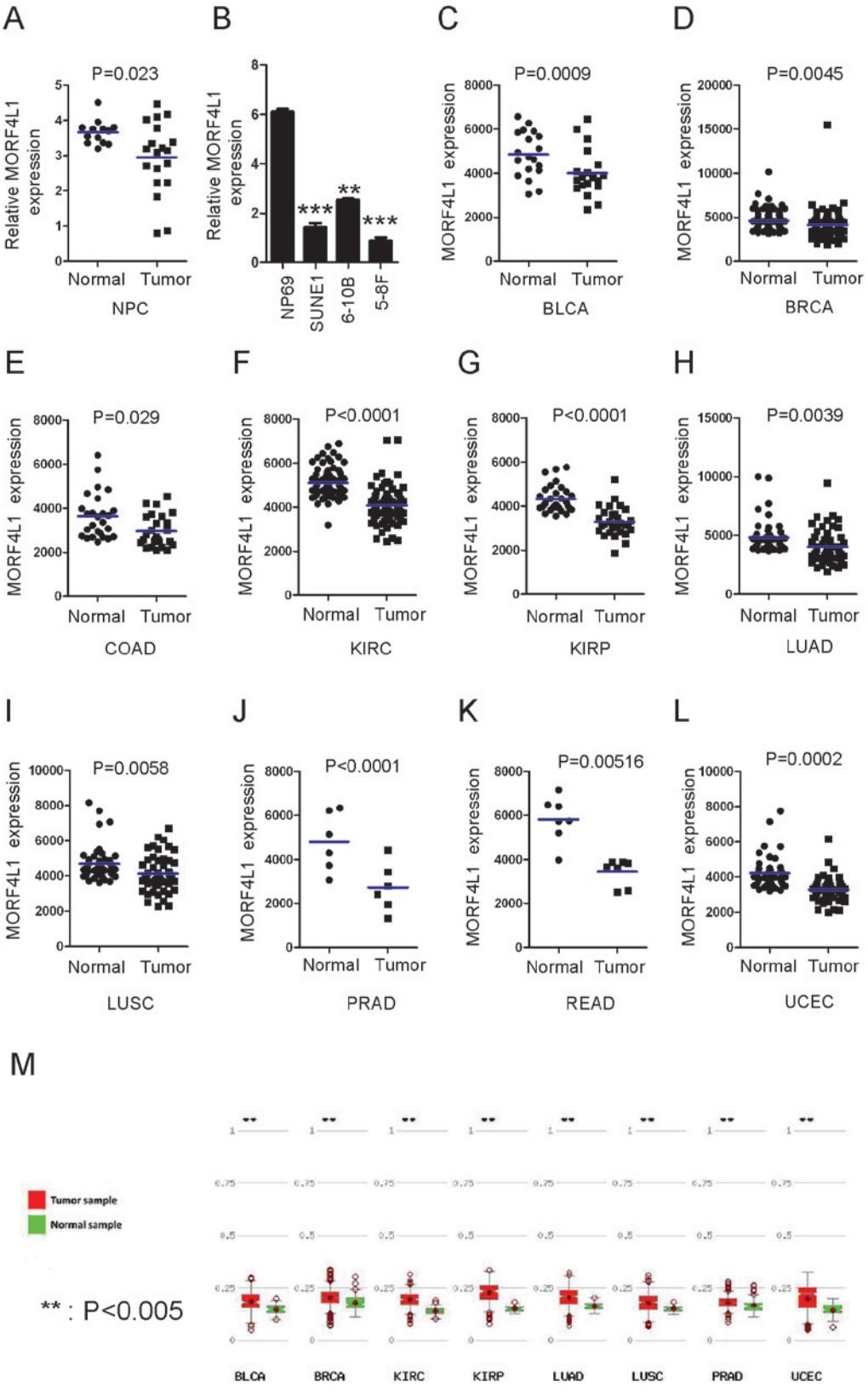

Figure 1. MORF4L1 expression is decreased in cancer. (A) RT-qPCR analysis of MORF4L1 expression in clinical NPC samples of both tumour (n=19) and normal tissues (n=13). Blue bars indicate mean values. (B) The mRNA levels of MORF4L1 in cell lines were determined by RT-qPCR. The mean ± SEM of NP69 group is 6.10±0.11, the mean ± SEM of 6-10B group 2.53±0.06; the mean ± SEM of SUNE1 group 1.42±0.17; the mean ± SEM of 5-8F group 0.88±0.13. **P<0.01 and ***P<0.001. Meta-analysis of MORF4L1 mRNA levels in clinical samples from the MethHC database in (C) BLCA, (D) BRCA, (E) COAD, (F) KIRC, (G) KIRP, (H) LUAD, (I) LUSC, (J) PRAD, (K) READ, (L) UCEC. (M) The methylation rate of MORF4L1 promoter is significantly higher in tumor cells, when compared with normal cells according to the online database (http://methhc.mbc.nctu.edu.tw/php/index.php). Blue bars indicate mean values. MORF4L1, Mortality factor 4-like 1; SEM, standard error; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; BLCA, bladder urothelial carcinoma, n=19; BRCA, breast invasive carcinoma, n=112; COAD, colon adenocarcinoma, n=26; KIRC, kidney renal clear cell carcinoma, n=72; KIRP, kidney renal papillary cell carcinoma, n=30; LUAD, lung adenocarcinoma, n=57; LUSC, lung squamous cell carcinoma, n=50; PRAD, prostate adenocarcinoma, n=50; READ, rectum adenocarcinoma, n=6; UCEC, uterine corpus endometrial carcinoma, n=7.

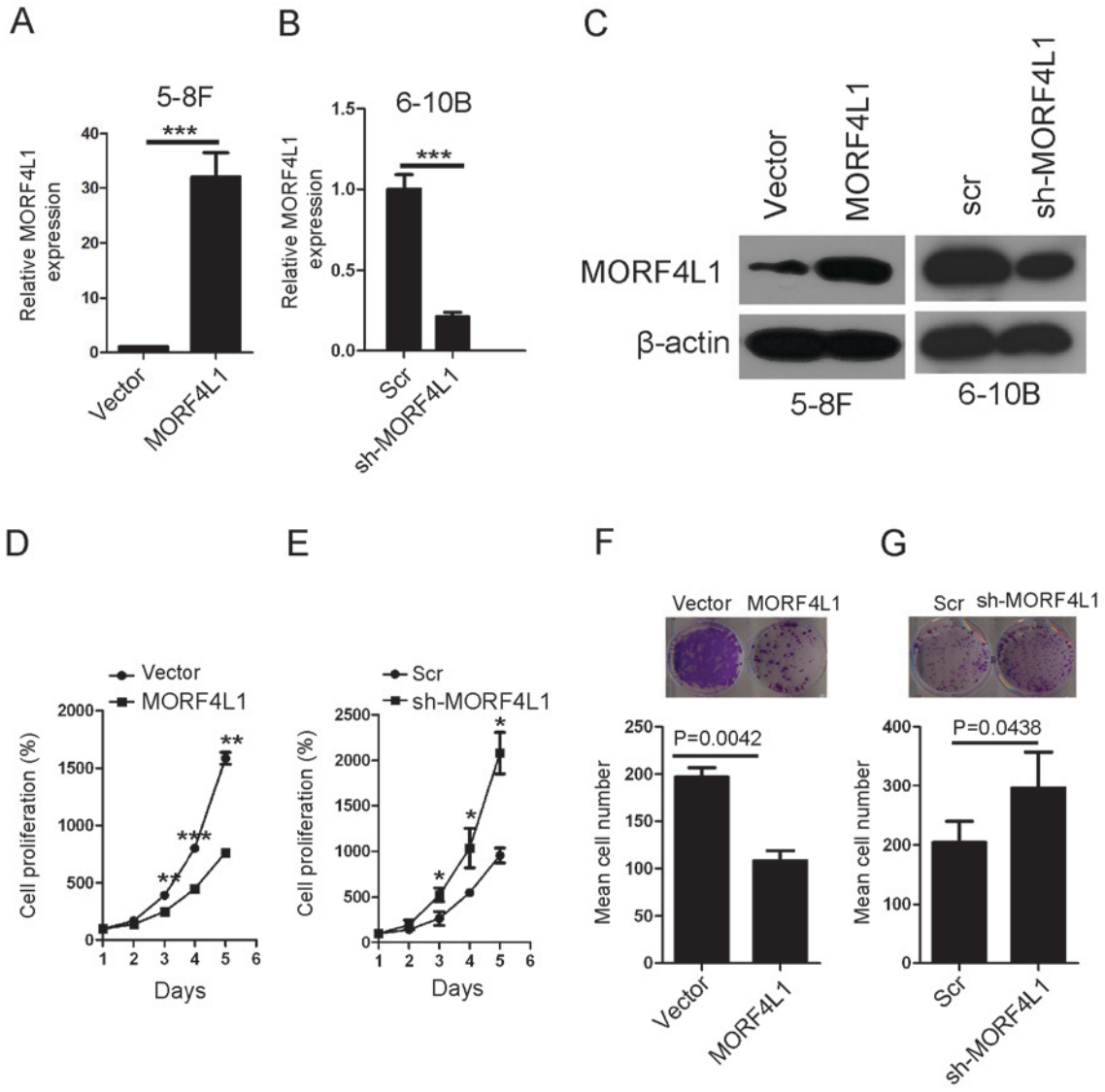

Figure 2. MORF4L1 inhibits NPC cell proliferation. The establishment of stable cell lines in (A) 5-8F and (B) 6-10B cells in which MORF4L1 was (A) over-expressed or (B) silenced was confirmed by RT-qPCR. GAPDH was used as the internal control. The bars represent the mean ± standard error values. (C) The generation of stable cell lines in 5-8F and 6-10B cells in which MORF4L1 was overexpressed or silenced was confirmed via western blotting. β-actin was used as the internal control. (D and E) Cell proliferation of the indicated stable cell lines *in vitro* was measured at different time points, using the CCK-8 assay. The bars represent mean ± standard error values, and the P-values were calculated using the Student's t-test. *P<0.05, **P<0.01 and ***P<0.001. (F and G) The colony formation ability of the indicated stable cell lines *in vitro* was measured at 14 days, as described in the Methods section. The bars represent mean ± standard error values, and the P-values were calculated using the paired Student's t-test. The top two images of F and G are representative images. MORF4L1, Mortality factor 4-like 1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; NPC, nasopharyngeal carcinoma; CCK-8, cell counting kit-8; Scr, scramble; sh-MORFL1, short hairpin RNA against MORFL1.

could be extended to other cancer types was subsequently investigated. MethHC is an integrated and beneficial database, which includes DNA methylation, gene expression, microRNA methylation and microRNA expression data, and the association between DNA methylation and gene expression from The Cancer Genome Atlas (25). As illustrated in Fig. 1C-L, with the data from MethHC, MORF4L1 was significantly downregulated in various types of cancer, including in breast, colon and lung cancer, suggesting that MORF4L1 expression is associated with cancer initiation or progression. To investigate the mechanisms underlying the reduction of MORF4L1 levels in cancer cells, the promoter sequence of MORF4L1 was analyzed and a CpG island was identified in this region. The methylation rate of the promoter was significantly higher in tumor cells, when compared with normal cells according to the online database (http://methhc.mbc.nctu.edu.tw/php/index.php) (Fig. 1M), which indicated that promoter hypermethylation may promote the downregulation of MORF4L1 in cancer types.

MORF4L1 inhibits cell proliferation and colony formation in NPC. Given that MORF4L1 expression is downregulated in NPC, the present study subsequently evaluated whether MORF4L1 is involved in regulating the biological behaviour of NPC cells. Based on the expression levels of MORF4L1 in NPC cells (Fig. 1B), stable cell lines with were constructed with ectopic expression of MORF4L1 (in 5-8F cells) or silenced MORF4L1 (in 6-10B cells) (Fig. 2A-C). The effects of MORF4L1 on the biological behaviour of these cell lines was subsequently investigated. Cell proliferation and colony formation assays indicated that the ectopic expression of

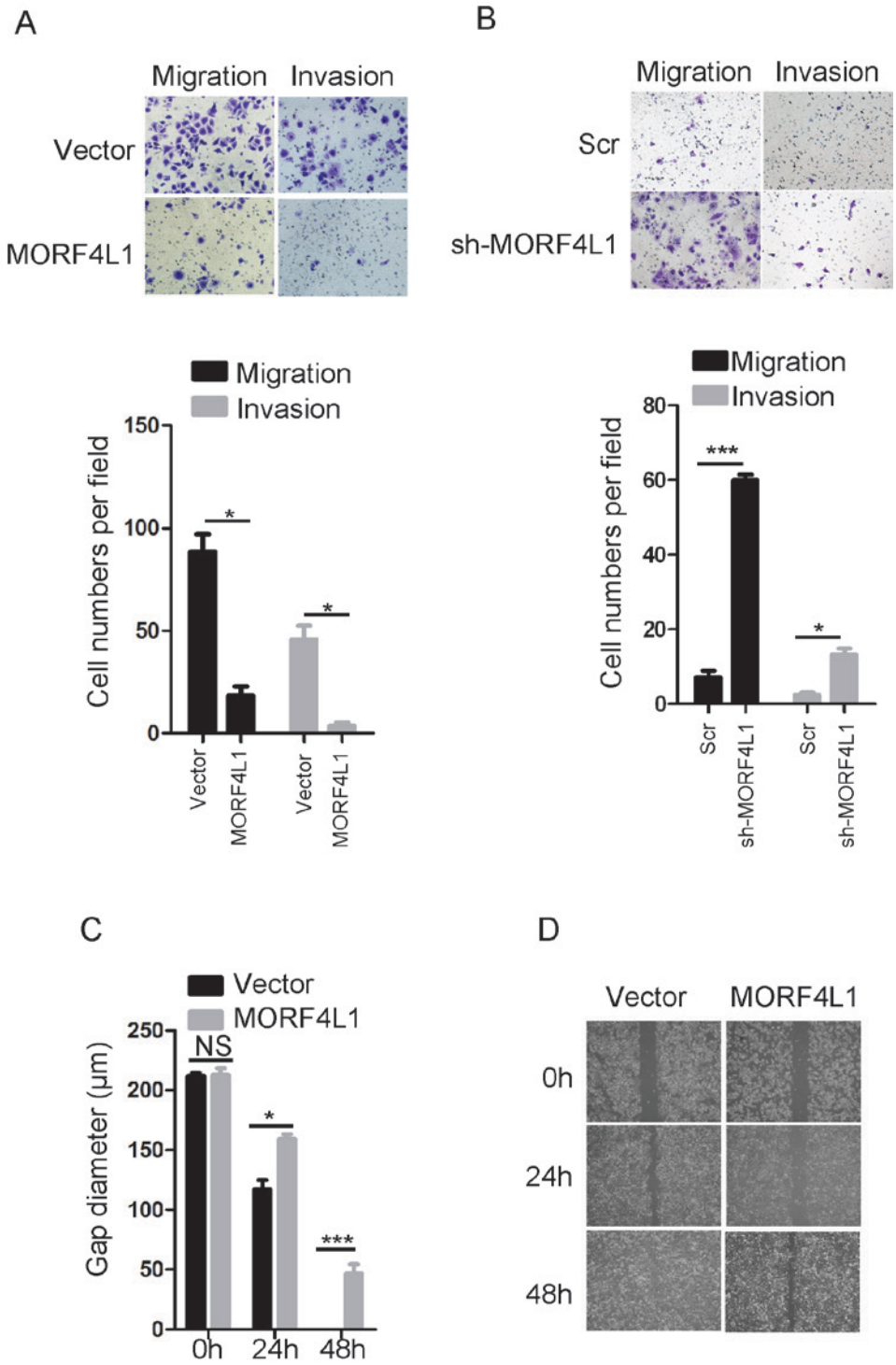

Figure 3. MORF4L1 suppresses the migration and invasion of NPC cells. The migration and invasion abilities of the (A) MORF4L1 and (B) sh-MORF4L1 cell lines were measured using the Transwell assay. The bars represent mean ± standard error values, and the P-values were calculated using paired Student's t-test. *P<0.05, ***P<0.001. Representative images are at x100 magnification. (C) Quantification and (D) representative images illustrating that gap closure was dramatically suppressed in cells overexpressing MORF4L1. Representative images are at magnification, x100. The bars represent mean ± standard error values. *P<0.05 and ***P<0.001. NS, nonsignificant; MORF4L1, Mortality factor 4-like 1; NPC, nasopharyngeal carcinoma; sh-MORF4L1, short hairpin RNA against MORFL1.

MORF4L1 significantly inhibited cell proliferation and colony formation abilities. Conversely, silencing MORF4L1 increased cell proliferation in 6-10B cells (Fig. 2D-G).

MORF4L1 inhibits NPC cell migration and invasion. The present study then examined whether MORF4L1 inhibited the migration and invasion of NPC cells. Using Transwell assays, a significant reduction in the number of migrated and invaded 5-8F cells following the overexpression of MORF4L1 (*P<0.05) (Fig. 3A), and an increased number of migrated and invaded 6-10B cells following MORF4L1 silencing were observed (*P<0.05, ***P<0.001; Fig. 3B). In addition, the ectopic

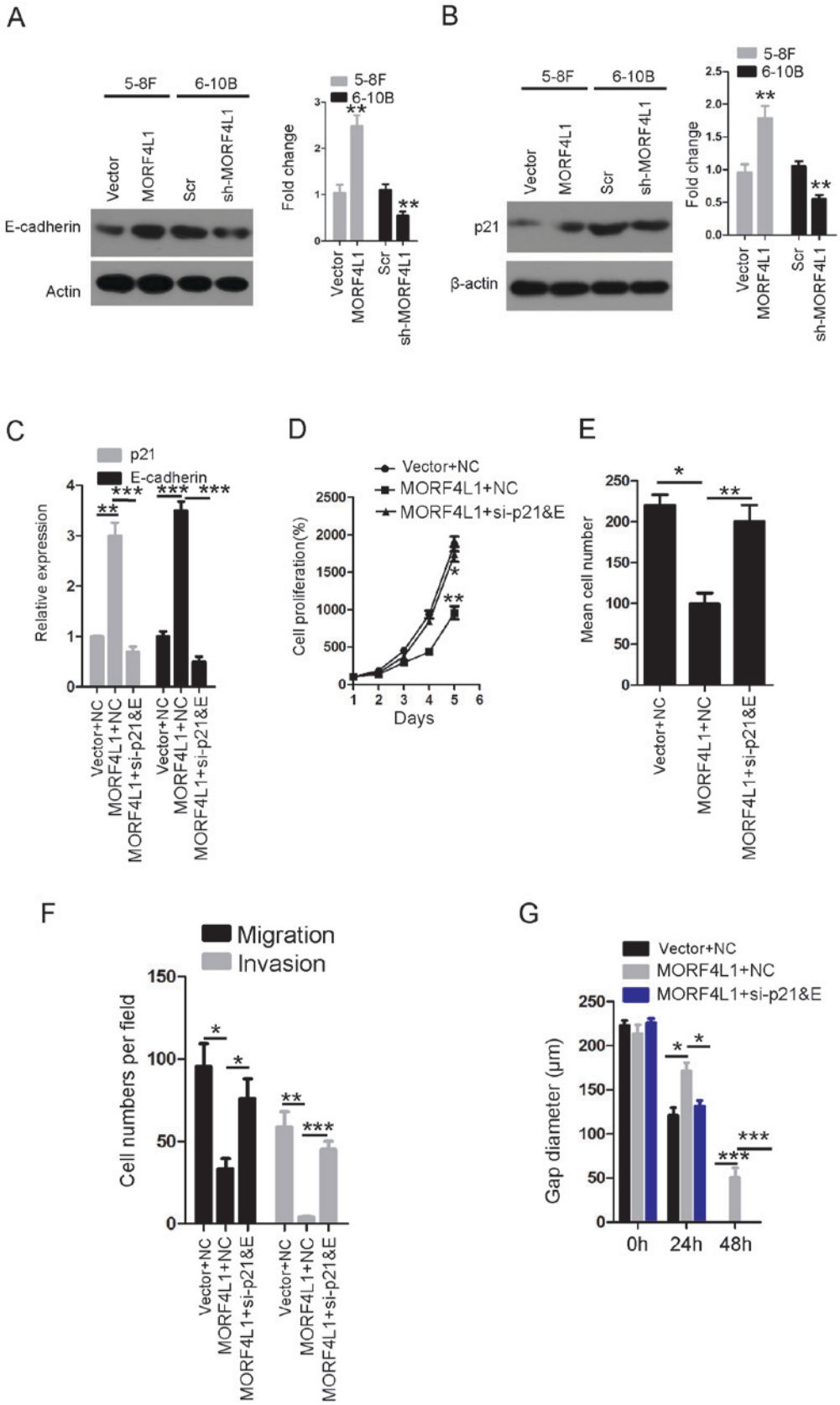

Figure 4. MORF4L1 increase sp21 and E-cadherin expression levels in NPC. Immunoblots of whole-cell lysates for (A) E-cadherin and (B) p21 from 5-8F and 6-10B cell lines. **P<0.01. (C) The levels of p21 and E-cadherin were detected via RT-qPCR. **P<0.01 and ***P<0.001. (D) Cell proliferation of the indicated cell lines *in vitro* was measured at different time points using a CCK-8 assay. The bars represent mean ± standard error values. *P<0.05 and **P<0.01 vs. Vector + NC. (E) The colony formation ability of the indicated cell lines *in vitro* was measured at 14 days, as described in the Methods section. The bars represent mean ± standard error values, *P<0.05 and **P<0.01. (F) The migration and invasion abilities of the indicated stable cell lines were measured using the Transwell assay. The bars represent mean ± standard error values. *P<0.05, **P<0.01 and ***P<0.001. (G) The ability of gap closure rescued following knockdown of p21 and E-cadherin in cells overexpressing MORF4L1. The bars represent mean ± standard error values. *P<0.05, **P<0.01 and ***P<0.001. NC, negative control; si-p21&E, p21 siRNA and E-cadherin siRNA; siRNA, small interfering RNA; MORF4L1, Mortality factor 4-like 1; NPC, nasopharyngeal carcinoma; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

expression of MORF4L1 significantly suppressed 5-8F cell wound healing after 24 h ($P<0.05$) and 48 h later ($P<0.001$) (Fig. 3C and D). Taken together, these findings indicated that MORF4L1 inhibited NPC cell migration and invasion.

MORF4L1 increases p21 and E-cadherin expression in NPC. In order to investigate the mechanisms underlying MORF4L1 suppressing cell proliferation, migration and invasion, the present study investigated the expression of p21 and E-cadherin. P21 and E-cadherin are key regulators in the progression of cancer, including NPC (26-29). As illustrated in Fig. 4A and B, p21 and E-cadherin protein levels were significantly higher in 5-8F cells that stably expressed ectopic MORF4L1. Conversely, silencing MORF4L1 in 6-10B cells significantly decreased the p21 and E-cadherin protein levels. To further verify MORF4L1 functions through p21 and E-cadherin, rescue experiments were performed with p21 siRNA and E-cadherin siRNA transfection (Fig. 4C). Compared with overexpression of MORF4L1 alone, the combined use of p21 siRNA and E-cadherin siRNA significantly increased the cancer cell proliferative (Fig. 4D), colony formation (Fig. 4E), migratory and invasive (Fig. 4F), and wound-healing abilities (Fig. 4G). These results suggest that MORF4L1 may regulate cell proliferation, migration and invasion by increasing the expression levels of p21 and E-cadherin in NPC.

Discussion

An improved understanding of the mechanisms underlying NPC development, progression and therapy resistance is required in order to design novel effective therapies for this cancer. The present study, to the best of our knowledge, demonstrated for the first time that MORF4L1 exhibits a key function in NPC cell proliferation, migration and invasion by upregulating the expression levels of p21 and E-cadherin.

Using RT-qPCR analysis and data mining, the present study demonstrated that the expression of MORF4L1 was significantly decreased in numerous types of cancer, including breast invasive carcinoma, colon adenocarcinoma and kidney renal clear cell carcinoma. In order to explore the mechanisms underlying the decreased expression of MORF4L1 in cancer cells, the methylation status of the MORF4L1 promoter was examined, as previous studies have reported that the hypermethylation of numerous tumour suppressor gene promoters leads to their decreased expression levels in cancer (30,31). The results of the present study demonstrated that the methylation rate of MORF4L1 promoter was higher in the majority of cancer tissues when compared with normal tissues, which suggests that promoter hypermethylation is one of the negative regulators of MORF4L1 in different types of cancer.

Chromatin structure may be dynamically altered by covalent modifications of histone tails through acetylation and methylation, which are involved in numerous important biological processes, including transcription, DNA replication and DNA damage repair (32). The results of the present study demonstrated that MORF4L1 increased the expression levels of p21 and E-cadherin to inhibit cell proliferation, migration and invasion. In summary, these findings demonstrate that MORF4L1 serves a role in tumour suppression by significantly increasing p21 and E-cadherin expression levels. However, there was little alteration to E-cadherin protein expression in the western blot analysis following MORF4L1 overexpression or knockdown. This may have been due to the transfection technique requiring further optimization to improve the level of knockdown or overexpression. In future studies, other methods of gene editing may be used, such as CRISPR, in order to improve knockdown. In addition, the cell lines in the present study are suitable for using in studying NPC, and their results *in vitro* are consistent with those *in vivo* (33-35). Together, these data provide novel insights into understanding the molecular basis underlying this malignancy.

Acknowledgements

Not applicable.

Funding

The present study was supported by National Science Foundation of China (grant no. 81660449 to YS; grant no. 81460430 to SWL; grant no. 81660452 to LZ) and Jiangxi Provincial Natural Science Foundation of China (grant no. 20161ACB21001, to YS; grant no. 20161BAB215188, to GH).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YS, RZ, LZ, GH and LS wrote the manuscript and performed experiments. SL, LX, KC and YP performed analysis and interpretation of data. All authors read and approved the final study.

Ethics approval and consent to participate

The use of human NPC tissues was reviewed and approved by the Ethical Committee of Jiangxi Cancer Hospital (Nanchang, China) and was performed in accordance with the approved guidelines. Written informed consent was obtained from all patients.

Patient consent for publication

Written informed consent was obtained from all patients for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

References

1. Sang Y, Chen MY, Luo D, Zhang RH, Wang L, Li M, Luo R, Qian CN, Shao JY, Zeng YX and Kang T: TEL2 suppresses metastasis by down-regulating SERPINE1 in nasopharyngeal carcinoma. Oncotarget 6: 29240-29253, 2015.
2. Wang L, Sang Y, Tang J, Zhang RH, Luo D, Chen M, Deng WG and Kang T: Down-regulation of prostate stem cell antigen (PSCA) by Slug promotes metastasis in nasopharyngeal carcinoma. J Pathol 237: 411-422, 2015.

3. Sang Y, Wang L, Tang JJ, Zhang MF, Zhang MX, Liu X, Zhang RH, Kang TB and Chen MY: Oncogenic roles of carbonic anhydrase IX in human nasopharyngeal carcinoma. Int J Clin Exp Pathol 7: 2942-2949, 2014.
4. Chen QY, Wen YF, Guo L, Liu H, Huang PY, Mo HY, Li NW, Xiang YQ, Luo DH, Qiu F, *et al*: Concurrent chemoradiotherapy vs radiotherapy alone in stage II nasopharyngeal carcinoma: Phase III randomized trial. J Natl Cancer Inst 103: 1761-1770, 2011.
5. Liu X, Lv XB, Wang XP, Sang Y, Xu S, Hu K, Wu M, Liang Y, Liu P, Tang J, *et al*: MiR-138 suppressed nasopharyngeal carcinoma growth and tumorigenesis by targeting the CCND1 oncogene. Cell Cycle 11: 2495-2506, 2012.
6. Li N, Feng L, Han HQ, Yuan J, Qi XK, Lian YF, Kuang BH, Zhang YC, Deng CC, Zhang HJ, *et al*: A novel Smac mimetic APG-1387 demonstrates potent antitumor activity in nasopharyngeal carcinoma cells by inducing apoptosis. Cancer Lett 381: 14-22, 2016.
7. Bei JX, Li Y, Jia WH, Feng BJ, Zhou G, Chen LZ, Feng QS, Low HQ, Zhang H, He F, *et al*: A genome-wide association study of nasopharyngeal carcinoma identifies three new susceptibility loci. Nat Genet 42: 599-603, 2010.
8. Ye SB, Zhang H, Cai TT, Liu YN, Ni JJ, He J, Peng JY, Chen QY, Mo HY, Jun-Cui, *et al*: Exosomal miR-24-3p impedes T-cell function by targeting FGF11 and serves as a potential prognostic biomarker for nasopharyngeal carcinoma. J Pathol 240: 329-340, 2016.
9. Wen X, Tang X, Li Y, Ren X, He Q, Yang X, Zhang J, Wang Y, Ma J and Liu N: Microarray expression profiling of long non-coding RNAs involved in nasopharyngeal carcinoma metastasis. Int J Mol Sci 17: 1956, 2016.
10. Li W, Li J, Wang Y, Zhang K, Li N, Tian Z, Ni B, Wang H and Ruan Z: Sphingosine kinase 1 is a potential therapeutic target for nasopharyngeal carcinoma. Oncotarget 7: 80586-80598, 2016.
11. Zhang L, Su B, Sun W, Li W, Luo M, Liu D, Mei Q, Long G, Hu G and Hu G: Twist1 promotes radioresistance in nasopharyngeal carcinoma. Oncotarget 7: 81332-81340, 2016.
12. Zhang P, Du J, Sun B, Dong X, Xu G, Zhou J, Huang Q, Liu Q, Hao Q and Ding J: Structure of human MRG15 chromo domain and its binding to Lys36-methylated histone H3. Nucleic Acids Res 34: 6621-6628, 2006.
13. Pardo PS, Leung JK, Lucchesi JC and Pereira-Smith OM: MRG15, a novel chromodomain protein, is present in two distinct multiprotein complexes involved in transcriptional activation. J Biol Chem 277: 50860-50866, 2002.
14. Bryce SD, Forsyth NR, Fitzsimmons SA, Clark LJ, Bertram MJ, Cuthbert AP, Newbold RF, Pereira-Smith OM and Parkinson EK: Genetic and functional analyses exclude mortality factor 4 (MORF4) as a keratinocyte senescence gene. Cancer Res 59: 2038-2040, 1999.
15. Yochum GS and Ayer DE: Role for the mortality factors MORF4, MRGX and MRG15 in transcriptional repression via associations with Pf1, mSin3A, and Transducin-Like Enhancer of Split. Mol Cell Biol 22: 7868-7876, 2002.
16. Chen Y, Li J, Dunn S, Xiong S, Chen W, Zhao Y, Chen BB, Mallampalli RK and Zou C: Histone deacetylase 2 (HDAC2) protein-dependent deacetylation of mortality factor 4-like 1 (MORF4L1) protein enhances its homodimerization. J Biol Chem 289: 7092-7098, 2014.
17. Tominaga K, Kirtane B, Jackson JG, Ikeno Y, Ikeda T, Hawks C, Smith JR, Matzuk MM and Pereira-Smith OM: MRG15 regulates embryonic development and cell proliferation. Mol Cell Biol 25: 2924-2937, 2005.
18. Zou C, Li J, Xiong S, Chen Y, Wu Q, Li X, Weathington NM, Han S, Snavely C, Chen BB and Mallampalli RK: Mortality factor 4 like 1 protein mediates epithelial cell death in a mouse model of pneumonia. Sci Transl Med 7: 311ra171, 2015.
19. Liang Y, Lin JC, Wang K, Chen YJ, Liu HH, Luan R, Jiang S, Che T, Zhao Y, Li de F, *et al*: A nuclear ligand MRG15 involved in the proapoptotic activity of medicinal fungal galectin AAL (Agrocybe aegerita lectin). Biochim Biophys Acta 1800: 474-480, 2010.
20. Garcia SN, Kirtane BM, Podlutsky AJ, Pereira-Smith OM and Tominaga K: Mrg15 null and heterozygous mouse embryonic fibroblasts exhibit DNA-repair defects post exposure to gamma ionizing radiation. FEBS Lett 581: 5275-5281, 2007.
21. Martrat G, Maxwell CM, Tominaga E, Porta-de-la-Riva M, Bonifaci N, Gómez-Baldó L, Bogliolo M, Lázaro C, Blanco I, Brunet J, *et al*: Exploring the link between MORF4L1 and risk of breast cancer. Breast Cancer Res 13: R40, 2011.
22. Yin Y, Zhong J, Li SW, Li JZ, Zhou M, Chen Y, Sang Y and Liu L: TRIM11, a direct target of miR-24-3p, promotes cell proliferation and inhibits apoptosis in colon cancer. Oncotarget 7: 86755-86765, 2016.
23. Lv XB, Liu L, Cheng C, Yu B, Xiong L, Hu K, Tang J, Zeng L and Sang Y: SUN2 exerts tumor suppressor functions by suppressing the Warburg effect in lung cancer. Sci Rep 5: 17940, 2015.
24. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
25. Huang WY, Hsu SD, Huang HY, Sun YM, Chou CH, Weng SL and Huang HD: MethHC: A database of DNA methylation and gene expression in human cancer. Nucleic Acids Res 43 (Database Issue): D856-D861, 2015.
26. Zhang RL, Peng LX, Yang JP, Zheng LS, Xie P, Wang MY, Huang BJ, Zhao HR, Bao YX and Qian CN: IL-8 suppresses E-cadherin expression in nasopharyngeal carcinoma cells by enhancing E-cadherin promoter DNA methylation. Int J Oncol 48: 207-214, 2016.
27. 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-beta. Oncotarget 6: 9781-9793, 2015.
28. Chen C, Lu Z, Yang J, Hao W, Qin Y, Wang H, Xie C and Xie R: MiR-17-5p promotes cancer cell proliferation and tumorigenesis in nasopharyngeal carcinoma by targeting p21. Cancer Med 5: 3489-3499, 2016.
29. Yi C, Wang Q, Wang L, Huang Y, Li L, Liu L, Zhou X, Xie G, Kang T, Wang H, *et al*: MiR-663, a microRNA targeting p21(WAF1/CIP1), promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma. Oncogene 31: 4421-4433, 2012.
30. Sang Y, Cheng C, Tang XF, Zhang MF and Lv XB: Hypermethylation of TET1 promoter is a new diagnosic marker for breast cancer metastasis. Asian Pac J Cancer Prev 16: 1197-1200, 2015.
31. Tang B, Li Y, Qi G, Yuan S, Wang Z, Yu S, Li B and He S: Clinicopathological significance of CDKN2A promoter hypermethylation frequency with pancreatic cancer. Sci Rep 5: 13563, 2015.
32. Chen M, Pereira-Smith OM and Tominaga K: Loss of the chromatin regulator MRG15 limits neural stem/progenitor cell proliferation via increased expression of the p21 Cdk inhibitor. Stem Cell Res 7: 75-88, 2011.
33. Chen J, Hao Y, Chen J, Huang L, Ao W, Yang J, Li L, Heng J, Chen Z, Liang W, *et al*: Colony stimulating factor-1 receptor promotes proliferation, migration and invasion in the human nasopharyngeal carcinoma 6-10B cell line via the phosphoinositide 3-kinase/Akt pathway. Oncol Lett 16: 1205-1211, 2018.
34. Nie GH, Li Z, Duan HF, Luo L, Hu HY, Yang WQ, Nie LP, Zhu RF, Chen XF and Zhang W: lncRNA C22orf32-1 contributes to the tumorigenesis of nasopharyngeal carcinoma. Oncol Lett 13: 4487-4492, 2017.
35. Yuan Y, Du Y, Hu XY, Liu MY, Du JK, Liu XM, Yu HE, Wang TZ, Pu JX, Zhong Q and Zou QF: Longikaurin A, a natural ent-kaurane, suppresses stemness in nasopharyngeal carcinoma cells. Oncology Lett 13: 1672-1680, 2017.