Overexpression of MCPH1 inhibits uncontrolled cell growth by promoting cell apoptosis and arresting the cell cycle in S and G2/M phase in lung cancer cells

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Abstract. Microcephalin (MCPH1/BRIT1) is a large nuclear protein that is involved in the early cellular response to DNA damage, the expression of which is reduced in a variety of types of human tumors. A recent study by our group demonstrated that MCPH1 expression is markedly decreased in lung cancer. However, it remains unclear whether inducing the expression of MCPH1 may ameliorate lung cancer, and, if so, which mechanisms underlie this process. The results of the present study demonstrated that MCPH1 expression was downregulated in lung cancer tissues compared with that in normal lung tissues. Furthermore, MCPH1 overexpression in A549 non-small cell lung carcinoma cells, successfully inhibited cell proliferation via arrest of the cell cycle in the S and G2/M phases. In addition, MCPH1 overexpression promoted cell apoptosis, in association with a significant increase in the quantities of Bax and active caspase-3, as well as a decrease in the level of Bcl-2. In conclusion the present results indicated that MCPH1 is involved in the regulation of apoptosis and entry into mitosis, suggesting that MCPH1 may function as a tumor suppressor and that it may be important in the pathogenesis of lung cancer.

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Introduction

Lung cancer is one of the most common types of malignancy (1). Worldwide, it remains the leading cause of cancer-related mortality in males and females, and was responsible for 1.56 million deaths annually in 2012 (2). Conventional treatments for lung cancer include surgery, chemotherapy and radiotherapy (3). Although these treatments produce marked benefits in patients with lung cancer, they have a range of side-effects, such as hair loss, immunosuppression, and nausea and vomiting. In addition, the prognosis of advanced lung cancer is very poor, and the five-year survival is ~16.8% in the US and lower still in the developing world (4,5). However, gene therapy, which involves the delivery of therapeutic DNA into a patient's cells, has been investigated as a novel treatment for lung cancer in a number of clinical trials. For example, Morgan *et al* (6) successfully treated metastatic melanoma in two patients by using killer T cells that had been genetically retargeted to attack cancer cells. Strategies for gene therapy include induction of apoptosis, tumor suppressor gene replacement, suicide gene expression, cytokine-based therapy, vaccination-based approaches and adoptive transfer of modified immune cells (7).

MCPH1, also termed *BRIT1* (BRCT-repeat inhibitor of hTERT expression), encodes the MCPH1 protein that contains three BRCT domains: one in the N-terminus and two in the C-terminus. Besides MCPH1, numerous other proteins that are involved in tumor suppression and the DNA damage response, such as BRCA1, BRCA2, 53BP1, XRCC1, Rad9, NBS1 and DNA polymerase λ, also contain BRCT domains (8). Previous studies have demonstrated that BRCT-containing MCPH1 may be important in maintaining genome stability, which requires the activation of cell cycle checkpoints and the repair of damaged DNA (9,10). Indeed, MCPH1 knockdown reduces the expression of BRCA1 and the checkpoint kinase, Chk1, in addition to NBS1 phosphorylation, resulting in intra-S and G2/M checkpoint loss (9).

Cancer development involves dysregulation of the expression of oncogenes and tumor suppressors. A number of DNA repair regulators have been associated with human cancer, such as BRCA1 and BRCA2 (11). Therefore, it is proposed that MCPH1, a key regulator of the DNA repair pathway and cell cycle checkpoints, may be involved in cancer development and progression. Indeed, recent studies have demonstrated that MCPH1 is downregulated in a variety of types of human cancer, including breast cancer (12,13), endometrial cancer (14), ovarian cancer (15), glioblastoma (16) and oral squamous cell carcinoma (17). Furthermore, a recent experiment using immunohistochemistry, conducted by our group, demonstrated that MCPH1 expression was markedly suppressed in lung cancer tissues (18). These results support the hypothesis that MCPH1 is a tumor suppressor gene. Thus, it was proposed that an increase in the expression of MCPH1 may be an effective therapy for human lung cancer.

The present study examined MCPH1 mRNA expression in human lung cancer tissues and normal lung tissues. The effect of increased MCPH1 expression on cell apoptosis and proliferation in the A549 non-small cell lung cancer cell line was subsequently investigated, in addition to the molecular mechanisms underlying this process.

Materials and methods

Lung cancer specimens. Lung Cancer specimens were obtained from 24 patients with lung cancer, who underwent surgery in the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) from July 2009 to June 2012. Normal adjacent tissues specimens were used as controls. These patients received neither chemotherapy nor radiotherapy prior to surgery. The acquisition and analysis of the lung cancer specimens was approved by the ethics committee of the hospital and the patients provided written informed consent.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from cell lines and tissue samples using TRIzol™ reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. Reverse transcription was performed at 42˚C for 30 min followed by inactivation at 94˚C for 5 min. The resultant first-strand cDNA was used as a template for PCR amplification. The cDNA was stored at -20˚C until use or immediately amplified by PCR in order to measure the expression of the genes of interest. The following oligonucleotide primers were used: Forward, 5'-CACCATCTTTCACTCACCTC-3' and reverse, 5'-CTTTACTGAGGAACTCCTGG-3' for MCPH1; and forward, 5'-ACCTGACCTGCCGTCTAGAA-3' and reverse, 5'-TCCACCACCCTGTTGCTGTA-3' for GAPDH. Each amplification program consisted of one cycle of 94˚C for 5 min, followed by 30 cycles of 94˚C for 10 sec, 60˚C for 30 sec and 72˚C for 30 sec. The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining (Sangon Biotech Co., Ltd., Shanghai, China). GAPDH was used as an internal control. Data analysis was performed using the $2^{-\Delta\Delta Ct}$ method.

Cell culture and transfection. A549 human lung cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen Life Technologies), penicillin (50 U/ml), and streptomycin (50 μg/ml; Gibco BRL, Grand Island, NY, USA). Cells were maintained at 37˚C in a humidified atmosphere of 5% CO_2. In order to increase MCPH1 expression, a pcDNA3.1 (-)MCPH1 plasmid was constructed. A fragment of human MCPH1 was amplified from the cDNA of HEK293T cells (Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) using specific primers (Forward, 5'-CACCATCTTTCACTCACCTC-3' and reverse, 5'-CTTTACTGAGGAACTCCTGG-3') with the *Hind*III and *Xho*I restriction sites. PCR was performed using a total of 1.0 μl cDNA and 0.25 μl Ex Taq™ Polymerase (Takara, Otsu, Japan) for 35 cycles of 94˚C for 1 min, 58˚C for 180 sec and 72˚C for 1 min, followed by 10 min at 72˚C. The PCR product (2,508 bp) was purified and digested with *Hind*III and *Xho*I, then cloned into a mammalian expression vector pcDNA3.1(-) with the corresponding restriction sites (Novagen, Darmstad, Germany). The recombinant plasmid was confirmed by DNA sequencing using the HiSeq 2000 Sequencing System (Illumina Inc, San Diego, CA, USA). Transient transfection was conducted using Lipofectamine 2000 (Invitrogen Life Technologies) for A549 cells, according to the manufacturer's instructions. Briefly, equal numbers of cells were plated in 24-well and 6-well plates and were grown to 80% confluence. Cells in the 24-well plates were transfected with 0.5 μg of pcDNA3.1(-)MCPH1 vector or pcDNA3.1 empty vector. Cells in the 6-well plates were tansfected with 1.0 μg of pcDNA3.1(-)MCPH1 vector or pcDNA3.1 empty vector. The indicated quantities of vectors were combined in Opti-MEM™ medium (Invitrogen Life Technologies) with Lipofectamine 2000. The solution was incubated for ~30 min at room temperature and then added to the cultured cells. After 4-6 h, the medium was changed for DMEM with 10% FBS.

MTT assay. A549 cells were transfected with pcDNA3.1 (-)MCPH1. At 96 h following transfection, 20 ml of MTT (5 mg/ml) was added to each well of a 96-well plate and the cells were cultured for 4 h. Cells were observed using a phase contrast microscope (TE2000; Nikon Corporation, Tokyo, Japan). The MTT was then discarded and 150 ml of dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) was added to each well. Absorbance was measured at 490 nm using a multi-well spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT, USA).

Flow cytometry analysis and apoptosis assay. Cell cycle distribution was determined using flow cytometry, following cell staining with propidium iodide (Sigma-Aldrich). In brief, floating and adherent cells were collected, washed with ice-cold phosphate-buffered saline and fixed with 70% ethanol. Cells were then treated with 50 μg/ml of RNase A (Sigma-Aldrich) and 50 μg/ml of propidium iodide for 30 min at room temperature. The stained cells were analyzed using flow cytometry (FACSCalibur™ flow cytometer; BD Biosciences, San Jose, CA, USA).

Western blot analysis. Cells were lysed in a lysis buffer containing 25 mM Tris-HCl, pH 7.5; 137 mM NaCl; 2.7 mM KCl; 1% Triton X-100; and protease inhibitor cocktail (Sigma-Aldrich).

Table I. Clinicopathological characteristics of patients with lung cancer in the present study.

Number	Gender	Age	Organ	Pathological diagnosis	Classification	TNM	Type
1	M	48	Lung	Adenocarcinoma	1	T2N0M0	Malignant
2	M	41	Lung	Adenocarcinoma	1	T2N0M0	Malignant
3	F	55	Lung	Adenocarcinoma	1	T2N1M0	Malignant
4	M	56	Lung	Adenocarcinoma	1	T1N0M0	Malignant
5	F	81	Lung	Adenocarcinoma	1	T2N1M0	Malignant
6	F	58	Lung	Papillary adenocarcinoma	1	T2N1M0	Malignant
7	M	44	Lung	Mucinous adenocarcinoma	2	T2NxM0	Malignant
8	M	32	Lung	Adenocarcinoma	1	T2N0M0	Malignant
9	F	35	Lung	Mucinous adenocarcinoma	2	T2N1M0	Malignant
10	M	42	Lung	Adenocarcinoma	2	T2N1M0	Malignant
11	F	63	Lung	Adenocarcinoma	1	T2N1M0	Malignant
12	F	31	Lung	Adenocarcinoma	3	T2N1M0	Malignant
13	F	55	Lung	Adenocarcinoma	3	T2N0M0	Malignant
14	M	64	Lung	Adenocarcinoma (sparse carcinoma infiltrating lung tissue)	3	T2N1M0	Malignant
15	M	64	Lung	Mucinous adenocarcinoma	3	T2N0M0	Malignant
16	M	52	Lung	Adenocarcinoma	3	T2NxM0	Malignant
17	M	71	Lung	Adenocarcinoma	3	T2N2M0	Malignant
18	M	70	Lung	Adenocarcinoma	3	T2NxM0	Malignant
19	F	66	Lung	Adenocarcinoma	3	T2N2M0	Malignant
20	M	54	Lung	Adenocarcinoma	3	T2N1M0	Malignant
21	F	35	Lung	Adenocarcinoma	3	T2N0M0	Malignant
22	M	53	Lung	Adenosquamous carcinoma	3	T2N1M0	Malignant
23	M	75	Lung	Squamous cell carcinoma	1	T2N1M0	Malignant
24	M	56	Lung	Squamous cell carcinoma	1	T2N2M0	Malignant

The protein concentration of cell lysates was determined using a Bio-Rad Protein Assay Kit I (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. Bovine serum albumin was used as a control. Equal quantities of cell lysates were separated by 10% SDS-polyacrylamide gel electrophoresis, electrotransferred onto Immobilon-P membrane filters (Millipore, Billerica, MA, USA) and blocked with 0.5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 at 4˚C. The membranes were incubated with anti-human mouse monoclonal IgG1 antibody to Bcl-2 (cat no. sc-509; 1:500 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit monoclonal IgG antibody to MCPH1 (cat no. ab123361; 1:200 dilution; Abcam, Cambridge, UK), rabbit monoclonal IgG antibody to β-actin (cat no. A0483; 1:100 dilution; Sigma-Aldrich), rabbit polyclonal IgG antibody to BAX (cat no. B3428; 1:2,000 dilution; Sigma-Aldrich) and rabbit polyclonal IgG antibody to caspase 3 (cat no. 9654; 1:500 dilution; Cell Signaling Technology, Inc., Danvers, MA, USA) at room temperature for 1 h, followed by incubation with horseradish peroxidase-conjugated secondary antibodies (anti-rabbit IgG, cat no. 7074P2, 1:3,000 dilution; and anti-mouse IgG, cat no. 7072S; 1:3,000 dilution; Cell Signaling Technology, Inc.) at room temperature for 1 h. Immunoreactive bands were visualized using an ECL system (GE Healthcare, Little Chalfont, UK), according to the manufacturer's instructions.

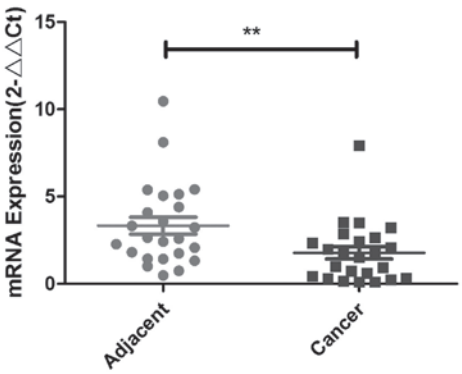

Figure 1. Expression of MCPH1 in patients with lung cancer. Reverse transcription-polymerase chain reaction demonstrated that the expression of MCPH1 mRNA was significantly reduced in lung cancer tissues compared with that in normal adjacent tissues. **P<0.01. MCPH1, microcephalin.

Statistical analysis. Data are presented as the mean ± standard error of the mean of ≥3 separate experiments. Multiple group comparisons were analyzed using one-way analysis of variance and post hoc Tukey's test, with plasmid treatment as the between-subjects factor. Paired Student's t-test was used to analyze the results of the RT-PCT assay. All statistical analyses were performed using the SPSS software (version 17.0; SPSS,

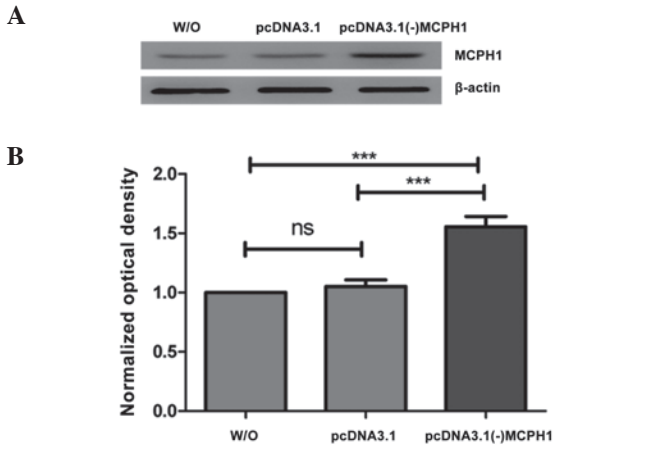

Figure 2. Induction of MCPH1 overexpression in the A549 lung cancer cell line. Immunoblotting of MCPH1 from A549 cells transfected with the pcDNA3.1 (-)MCPH1 plasmid (pcDNA3.1 (-)MCPH1), the empty vector, pcDNA3.1 and in the W/O group. (A) Representative western blots. (B) MCPH1 expression was significantly increased in A549 cells transfected with pcDNA3.1 (-)MCPH1 compared with pcDNA3.1-transfected cells and the W/O group (n=3). One-way analysis of variance was used: F (2,6)=79.242; P<0.001. ***P<0.001. MCPH1, microcephalin; W/O, cells not transfected with pcDNA3.1(-) MCPH1 or the empty vector; ns, no significant difference.

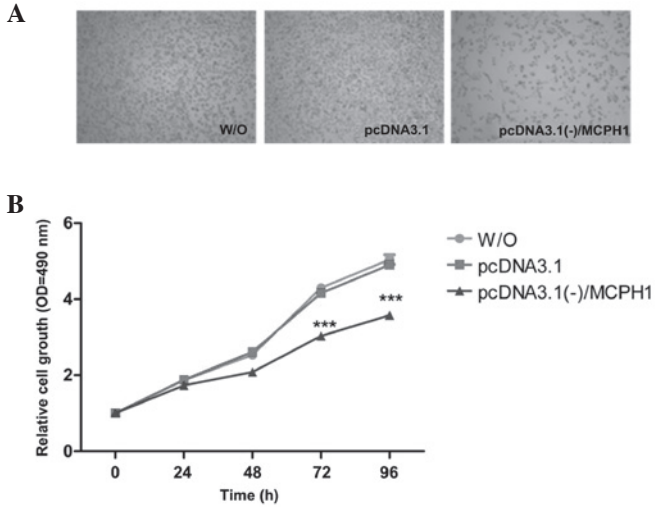

Figure 3. MCPH1 overexpression inhibited cell proliferation in A549 cells. At 96 h following transfection, cells were observed using a phase contrast microscope. (A) Representative cell morphology. (B) Growth curves of each group. Cell proliferation was significantly inhibited in A549 cells transfected with pcDNA3.1 (-)MCPH1 compared with those transfected with pcDNA3.1 and with the W/O control group (n=3). One-way analysis of variance was used: F (2,6)=241.767; P<0.001. ***P<0.001 vs. pcDNA3.1 or W/O. MCPH1, microcephalin; W/O, cells not transfected with pcDNA3.1(-) MCPH1 or the empty vector; ns, no significant difference.

Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

MCPH1 mRNA is downregulated in lung cancer tissues. It has recently been reported that MCPH1 expression is markedly decreased in lung cancer tissues (18). In order to confirm this results, the present study measured MCPH1 mRNA expression in lung cancer tissues and normal adjacent tissues, using RT-PCR. The clinicopathological characteristics of the 24 patients with lung cancer are presented in Table I. The results demonstrated that MCPH1 mRNA expression was significantly reduced in lung cancer tissues compared with that in normal adjacent tissues (P=0.008; Fig. 1).

MCPH1 overexpression inhibits cell proliferation in A549 lung cancer cells. As MCPH1 has been shown to be involved in the activation of cell cycle checkpoints (18) and its expression is markedly reduced in lung cancer (Fig. 1), the present study aimed to determine whether overexpression of MCPH1 in the A549 cell line may inhibit uncontrolled cell growth. In order to increase MCPH1 expression, a pcDNA3.1 (-)MCPH1 plasmid was constructed. It was observed that A549 cells transfected with the pcDNA3.1 (-)MCPH1 plasmid exhibited significantly increased MCPH1 expression, compared with those transfected with the empty vector pcDNA3.1 (P=0.589; Fig. 2A and B) or with the untreated control.

Subsequently, the effect of MCPH1 overexpression on the proliferation of the A549 cell line was examined. The results from the MTT assay demonstrated that A549 cells transfected with the pcDNA3.1 (-)MCPH1 plasmid exhibited significantly reduced cell proliferation compared with cells transfected with the empty vector (P=0.145) or with cells not transfected with pcDNA3.1(-) MCPH1 or the empty vector (P<0.001; Fig. 3A and B).

A previous study has demonstrated that MCPH1 may regulate the S phase and G2/M cell cycle checkpoints in breast cancer (19). Therefore, the present study sought to determine whether MCPH1 overexpression results in S phase and G2 phase arrest, and subsequently inhibits uncontrolled cell growth in A549 cells. The results of the flow cytometry assay demonstrated that transfection with pcDNA3.1 (-)MCPH1 resulted in a significant increase in the proportion of A549 cells in S phase (11.6700±0.352%) compared with untreated control cells (9.8567±0.06936%; P=0.006) and cells transfected with pcDNA3.1 (10.287±0.388%; P=0.018 Fig. 4A-C). In addition, transfection with pcDNA3.1 (-)MCPH1, resulted in a significant increase in the proportion of cells in the G2/M phase (11.817±0.2980%) compared with untreated control cells (6.133±0.227%; P<.0001) and with cells transfected with pcDNA3.1 (6.953±0.2773%; P<0.001; Fig. 4A, B and D).

These results suggested that overexpression of MCPH1 leads to G2/M and S phase cell cycle arrest, and subsequently inhibits cell proliferation in the A549 lung cancer cell line.

MCPH1 overexpression promotes cell apoptosis in the A549 cell line. In addition to uncontrolled cell growth, a further characteristic of cancerous cells is the evasion of apoptosis (20,21). Therefore, the present study sought to determine whether MCPH1 overexpression may promote cell apoptosis in addition to inhibiting cell proliferation.

In order to evaluate the effect of MCPH1 overexpression on cell apoptosis, flow cytometry analysis was conducted. The results of this analysis demonstrated that A549 cells transfected with the pcDNA3.1 (-)MCPH1 plasmid exhibited a significant increase in the rate of apoptosis compared with untreated control cells and cells transfected with pcDNA3.1 (both P<0.001; Fig. 5A and B).

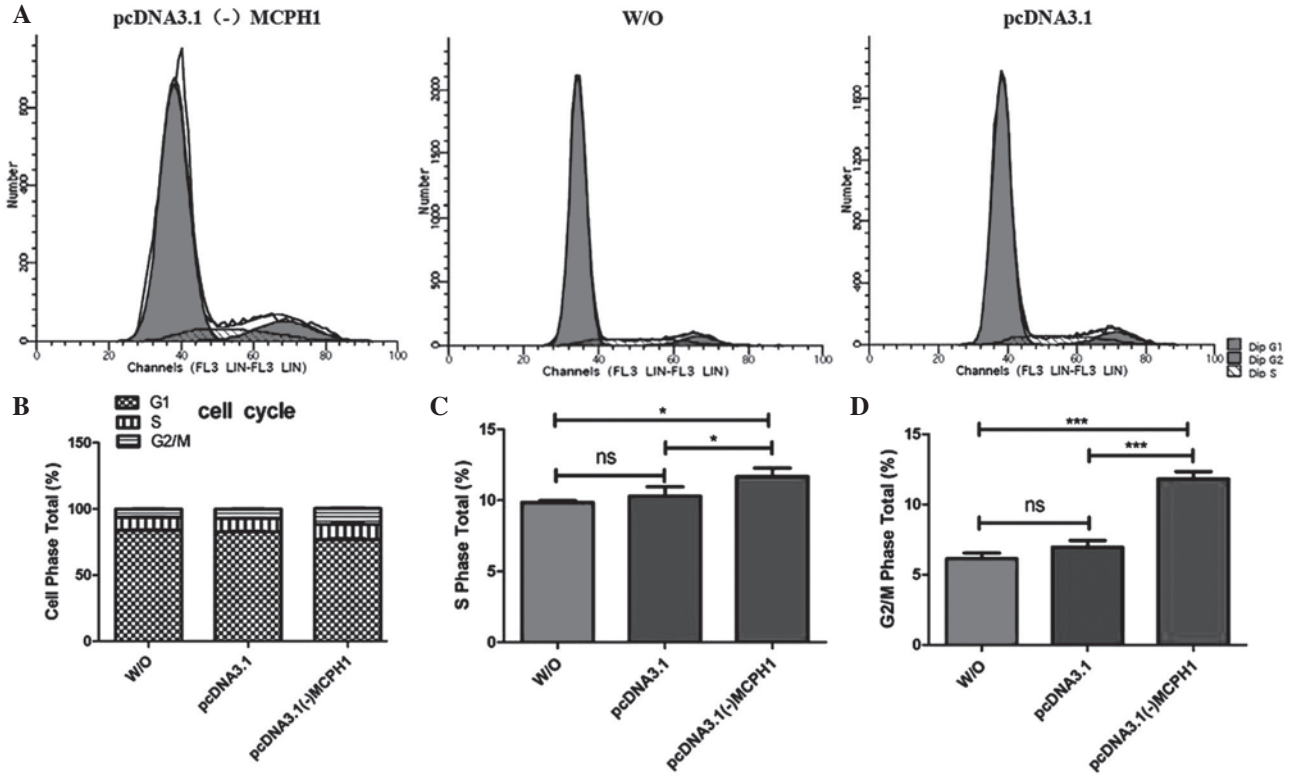

Figure 4. MCPH1 overexpression arrested the cell cycle at the S and G2/M phases. (A) and (B) Flow cytometric analysis demonstrated that MCPH1 overexpression altered cell cycle distribution in A549 cells (n=3). (C) and (D) MCPH1 overexpression significantly increased the proportion of cells in S phase (C) and G2/M phase (D). One-way analysis of variance was used: F (2,6)=9.647; P=0.013 for S phase and F (2,6)=130.351; P<0.001 for G2/M phase. *P<0.05 and ***P<0.001. MCPH1, microcephalin; W/O, cells not transfected with pcDNA3.1(-) MCPH1 or the empty vector; ns, no significant difference.

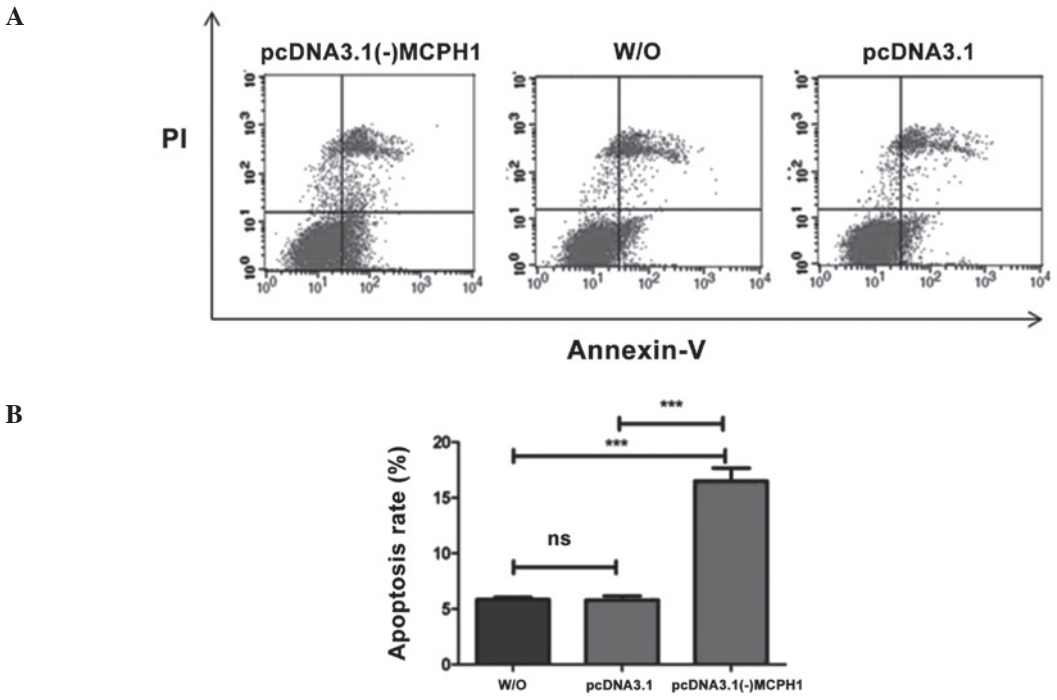

Figure 5. MCPH1 overexpression promoted cell apoptosis. (A) Representative cell cycle distribution in A549 cells. (B) Flow cytometric analysis demonstrated that cell apoptosis was significantly increased in A549 cells transfected with pcDNA3.1 (-)MCPH1 compared with cells transfected with pcDNA3.1 and the W/O control (n=3). One-way analysis of variance was used: F (2,6)=219.201; P<0.001. ***P<0.001. MCPH1, microcephalin; W/O, cells not transfected with pcDNA3.1(-) MCPH1 or the empty vector; ns, no significant difference.

Cell apoptosis is known to be associated with alterations in the expression of certain genes, such as caspase-3, Bax and Bcl-2. Therefore, the present study measured changes in the expression of these proteins, following MCPH1 overexpression

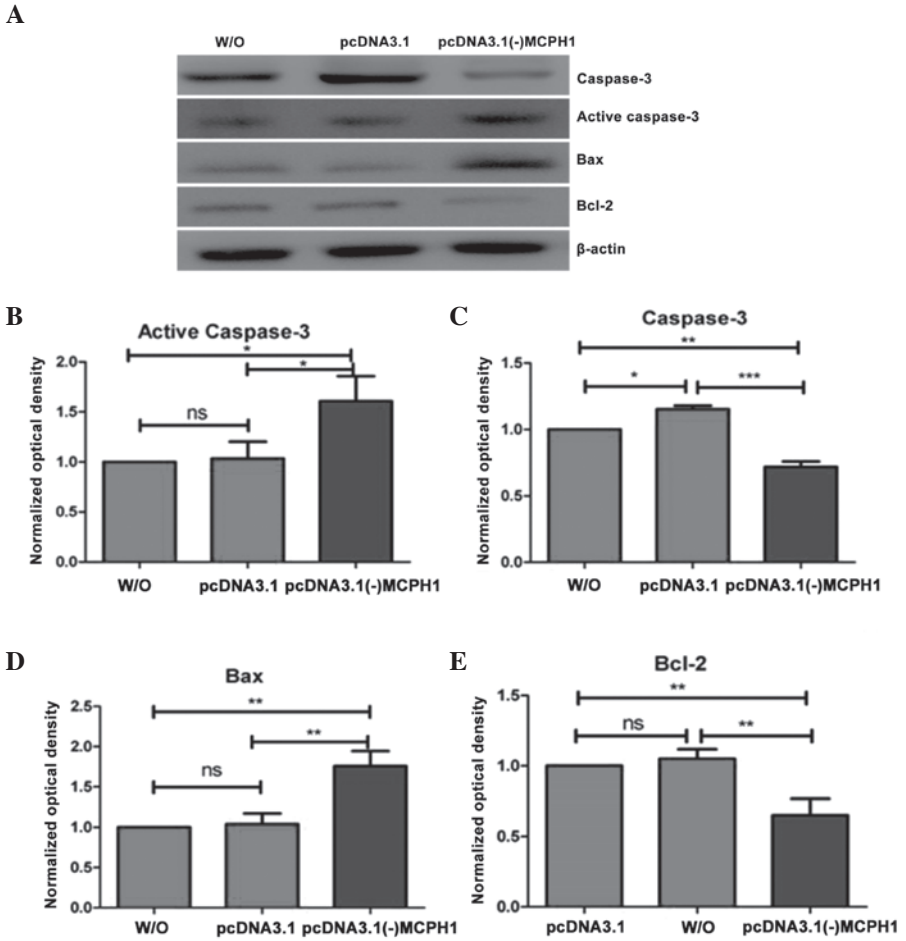

Figure 6. MCPH1 overexpression reduced the Bcl-2/Bax ratio and increased active caspase-3 expression in A549 cells. (A) Western blot analysis of the effects of MCPH1 overexpression on caspase-3, Bax and Bcl-2 expression. (B) and (C) A549 cells transfected with pcDNA3.1 (-)MCPH1 exhibited significantly increased expression of active caspase-3 (B) and decreased expression of inactive caspase-3 (C), compared with those transfected with pcDNA3.1 and with the W/O control group (n=3). (D) and (E) A549 cells transfected with pcDNA3.1 (-)MCPH1 exhibited significantly increased expression of Bax (D) and decreased expression of Bcl-2 (E) compared with those transfected pcDNA3.1 and with the W/O control group (n=3). One-way analysis of variance was used: F (2,6)=11.52, P=0.009 for active caspase-3; F (2,6)=60.278, P<0.001 for caspase-3; F (2,6)=30.979, P=0.001 for Bax; and F (2,6)=22.922, P=0.001 for Bcl-2. *P<0.05, **P<0.01 and ***P<0.001. MCPH1, microcephalin; W/O, cells not transfected with pcDNA3.1(-) MCPH1 or the empty vector; ns, no significant difference.

in A549 cells. The results demonstrated that the expression of active caspase-3 was significantly increased in cells transfected with pcDNA3.1 (-)MCPH1 compared with untreated control cells and cells transfected with pDNA3.1 (P=0.012 and P=0.016, respectively; Fig. 6A and B), while the expression of inactive caspase-3 was significantly decreased in A549 cells transfected with the pcDNA3.1 (-)MCPH1 plasmid, compared with untreated control cells and cells treated with pcDNA3.1 (P=0.001 and P<0.001, respectively; Fig. 6A and C). A549 cells transfected with the empty vector, pcDNA3.1, exhibited an increase in the expression of active caspase-3 compared with untreated control cells (P=0.021; Fig. 6A and C). However, there was no significant difference in the expression of active caspase-3 in this group compared with the untreated control cells (P=0.970; Fig. 6A and B). Furthermore, the expression of Bax was significantly increased in A549 cells that had been transfected with the pcDNA3.1 (-)MCPH1 plasmid compared with untreated control cells and cells transfected with the empty vector (both P=0.001; Fig. 6A and D). There was no significant difference in Bax expression between the untreated control cells and the cells transfected with the empty vector (P=0.932; Fig. 6A and D). By contrast, the expression of Bcl-2, an antiapoptotic protein, was significantly reduced in A549 cells that were transfected with the pcDNA3.1 (-)MCPH1 plasmid, compared with that in untreated control cells and cells transfected with pcDNA3.1 (P=0.004 and P=0.002, respectively; Fig. 6A and E). The expression of Bcl-2 in cells transfected with pcDNA3.1 was not significantly different from that in the untreated control cells (P=0.741; Fig. 6A and E). These results indicated that overexpression of MCPH1 may suppress tumorigenesis via the promotion of cell apoptosis.

Discussion

The present study confirmed that MCPH1 expression is downregulated in human lung cancer, and demonstrated that an increase in MCPH1 expression *in vitro* suppresses uncontrolled cell proliferation and promotes cell apoptosis. MCPH1 may therefore be involved in the pathogenesis of lung cancer.

Increasing evidence has suggested that MCPH1 expression is associated with the development of a number of types of cancer, such as breast cancer (12,13), endometrial cancer (14), ovarian cancer (15), glioblastoma (16) and oral squamous cell carcinoma (17). A more recent study reported that

knockdown of MCPH1 in mice leads to genomic instability and may enhance cancer susceptibility (10). In accordance with the results of a recent study conducted by our group (18), the present study demonstrated that the expression of MCPH1 mRNA was significantly downregulated in human lung cancer tissues (Fig. 1). Furthermore, induced overexpression of MCPH1 in A549 non-small cell lung cancer cells resulted in the suppression of cell proliferation (Fig. 3) and an increase in cell apoptosis (Fig. 5).

Previous studies have reported that MCPH1 is required for DNA damage-induced intra-S and G2/M checkpoints, and that this requirement may in part result from its regulation of the expression of BRCA1 and Chk1 (9,19). Therefore, MCPH1 downregulation in lung cancer tissues may lead to loss of the intra-S phase and G2/M phase checkpoints. In the current study, MCPH1 overexpression in A549 cells arrested the cell cycle in the S and G2/M phases (Fig. 4), and subsequently inhibited cell proliferation. The precise mechanisms underlying this effect remain to be elucidated, although they may involve the inhibitory effects of MCPH1 on cyclins and related cyclin-dependent kinase (CDK) enzymes, as a recent study reported that overexpression of MCPH1 decreases the expression of cyclin A2 and cyclin B1 in cervical cancer cells (22). Thus, further investigation of the effects of cyclin and CDK in lung cancer cells, with or without MCPH1 overexpression, may help to determine whether the inhibitory effect of MCPH1 on lung cancer cell proliferation is a result of its inhibitory effect on cyclin and CDK expression.

In addition to cell proliferation, cell apoptosis may also contribute to the inhibition of uncontrolled cell growth in lung cancer cells that are overexpressing MCPH1. A recent study reported that MCPH1 is involved in E2F1-mediated apoptosis; knockdown of MCPH1 in HEK293 cells resulted in decreased expression of E2F1 target genes, such as caspase-3, caspase-7, BRCA1 and Chk1 (23), which are important in cell apoptosis. Mitochondria participate in the intrinsic apoptotic pathway and tumors arise more frequently through the intrinsic pathway than the extrinsic pathway as a result of the sensitivity of the intrinsic pathway to apoptosis (24). In the intrinsic pathway, apoptosis is mediated by proteins in the Bcl-2 family. Alterations in the equilibrium between Bcl-2 and Bax lead to increased permeabilization of the mitochondrial outer membrane, with consequent cytochrome *c* release and ultimately activation of the caspase cascades (25,26). In the present study, MCPH1 overexpression was shown to induce cell apoptosis in human lung cancer cells by flow cytometric analysis (Fig. 5). Furthermore, MCPH1 overexpression reduced the Bcl-2/Bax ratio, as reflected by an increase in the expression of Bax and a decrease in that of Bcl-2 (Fig. 6), indicating that mitochondrial-mediated apoptosis had been initiated. In addition, caspase-3 was activated by MCPH1 overexpression (Fig. 6), which suggested that MCPH1 induces caspase-associated cell apoptosis. Therefore, overexpression of MCPH1 may induce cell apoptosis in lung cancer cells via caspase activation-dependent induction of the intrinsic apoptotic pathway.

In conclusion, MCPH1 expression was reduced in human lung cancer tissue in comparison with normal adjacent tissues, and overexpression of MCPH1 inhibited uncontrolled lung cancer cell growth via induction of cell cycle arrest at S phase and G2/M phase, and promotion of mitochondrial apoptosis. These findings suggest that MCPH1 may function as a tumor suppressor gene, and that it may be involved in the development and progression of human lung cancer. Furthermore, inducing an increase in MCPH1 expression may have potential as a therapeutic approach in lung cancer.

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