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Original Article

MTERF1 regulates the oxidative phosphorylation activity and cell proliferation in HeLa cells

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The mitochondrial transcription termination factor (MTERF) family is a group of highly conserved DNA-binding proteins composed of four key members, MTERF1–4. To date, several studies have investigated the binding sites of MTERF1 on mitochondrial genome and the regulation of mitochondrial gene transcription, but the more intricate connection between mitochondrial genes transcription regulation, mitochondrial oxidative phosphorylation (OXPHOS), and cell proliferation is still poorly understood. In this study, we constructed over-expression and knockdown vectors of *MTERF1* that were transfected into HeLa cells to investigate the functions of MTERF1. Results showed that although MTERF1 is a positive regulatory factor of mitochondrial genes transcription, it had no significant effect on the replication of mitochondrial DNA. Over-expression of MTERF1 increased mitochondrial oxidative phosphorylation activity and promoted ATP synthesis, cyclin D1 expression, and cell proliferation, while its knockdown inhibited ATP synthesis, decreased cyclin D1 expression, and slowed the cell growth. These results suggested that MTERF1 may promote cell proliferation by regulating oxidative phosphorylation activity in HeLa cells. Ultimately, these findings create a foundation for further and more conclusive studies on the physiological functions of MTERF family by providing novel insights into the potential mechanisms underlying cell proliferation regulation.

Keywords mitochondrial transcription termination factor 1; mitochondrial genes transcription; oxidative phosphorylation; cell proliferation

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Introduction

Mitochondrial oxidative phosphorylation (OXPHOS) provides the majority of the cellular ATP. Given ATP's role as the foundation for all cellular energy, gaining a clearer understanding of the regulation of oxidative phosphorylation function is particularly important. There are 101 known genes involved in the five complexes of OXPHOS, the majority of which are encoded in the nucleus with 13 catalytically important proteins encoded by the mitochondrial DNA (mtDNA) [1]. Mitochondrial gene transcription itself is completely regulated by nuclear gene-encoded proteins with the basal mtDNA transcription components consisting of mitochondrial RNA polymerase (POLRMT), transcription initiation factor A (TFAM), mitochondrial transcription initiation factor B1 or B2 (TFB1/2), and transcription termination factors. Together, these elements are sufficient to initiate transcription *in vitro* [2].

Though several mtDNA transcription regulatory proteins have been identified to date, the *in vivo* regulation of mitochondrial transcription and OXPHOS function remains poorly understood [3,4]. The mitochondrial transcription termination factor (MTERF) family is a member of the mitochondrial transcription regulatory factors. The MTERF family is composed of four members, known as MTERF1–4 [5]. The mitochondrial transcription termination factor 1 (MTERF1) is an important mtDNA-binding protein [6]. Previous structural analysis has shown that MTERF1 contains two mtDNA-binding domains and three leucine-zipper regions with multiple binding sites in the human mitochondrial genome [6–10]. Moreover, MTERF1 is a positive mitochondrial transcription regulation protein [11]. MTERF2, meanwhile, is a serum-inhibitory protein that binds to the mtDNA promoter, and regulates OXPHOS by modulating mtDNA transcription [1,8]. Recent studies have demonstrated that MTERF2 in combination with the mtDNA is not the specific negative regulation of mtDNA replication level [11,12]. Mammalian MTERF3 is thought to be a negative regulator of mtDNA transcription by binding to the mtDNA promoter region, and inhibiting the mtDNA transcription, slowing down cell energy production [13,14]. MTERF4 is likewise reported to be the last member of the MTERF family, locating

on the mtDNA, acting to maintain normal cell growth [15,16]. Although different family members have similar structures, not all of the members display the regulation of transcription termination function [17].

Most recent studies on mitochondrial transcription termination factors primarily focused on the combination of mtDNA and transcriptional regulation of mitochondrial genes [6,7,9,12,13], but there has been no clear connection made between mitochondrial genes transcription regulation, mitochondrial OXPHOS, and cell proliferation. In this study, we used over-expressed and knockdown variants of *MTERF1* to gain a clearer picture of MTERF1's primary functions. Our results showed that MTERF1 played an essential role in regulating mammalian cell proliferation. This functional identification of MTERF1 may greatly help to clarify the overall physiological function of MTERF family, and provides some new insights into the underlying mechanisms of cell cycle regulation.

Materials and Methods

Plasmid construction

Total RNA was extracted from HeLa cells using TRIzol (Life Technologies, Foster City, USA), after which cDNA was synthesized using the SuperScript First-Strand Kit (Invitrogen, Carlsbad, USA). Primers to amplify the open reading frame (ORF) of *MTERF1* were designed based on the known human *MTERF1* nucleotide sequences deposited within GenBank (accession no. NM_006980.3) as follows: forward: 5′-AAGCTTGCCACCATGCAGAGCC-3′; reverse: 5′-GGATCCGGCAAATCTGCTTAACTTT-3′. Polymerase chain reaction (PCR) was performed as follows: 1 cycle at 94°C for 3 min, 30 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 2 min, followed by 1 cycle at 72°C for 10 min. The resulting PCR products were separated by agarose gel electrophoresis and then purified with Gel Extraction Kit (Omega, Norcross, USA). Agarose-purified PCR fragments were cloned into pMD-19T vector (TakaRa, Dalian, China) and transfected into *Escherichia coli* DH5α competent cell (TransGen, Beijing, China), and then finally identified by plasmid-DNA sequencing with M13 sequencing primers (Sangon, Shanghai, China). The *MTERF1* ORF was then cloned into p3×Flag expression vector (Sigma, St Louis, USA) with *Hin*dIII and *Bam*HI restriction enzyme sites to generate a recombinant plasmid named MTERF1-Flag.

For *MTERF1* gene knockdown, we constructed two *MTERF1* siRNA expression vectors. Initially, the mRNA sequence of *MTERF1* (NM_006980.3) was submitted to Oligoengine RNAi DESIGN Center (http://www.oligoengine.com) to find optimal target nucleotide sequences. We selected two target sequences from these target nucleotide sequences to construct interference plasmids. The target nucleotide sequence 472–493 (5′-AACACGTACTCCCGAGAATCT-3′) and the sequence 343–364 (5′-AAGGAAACGACAGCC TGGAGT-3′) were designed as siRNA template. The siRNA oligonucleotides were annealed to form the insert fragments which were then cloned into pSilencer^{TM} 4.1-CMV neo (Ambion, Austin, USA). The recombinant plasmids were named pSi1-MTERF1 (also named as pSi-MTERF1) and pSi2-MTERF1, respectively. The pSilencer 4.1-CMV neo Negative Control (Ambion) was used as a negative control, named NK-pSi.

Cell culture and transfection

HeLa cells kindly provided by Dr Guoyang Liao from the Kunming Biological Institute of Medical Science, Chinese Academy of Medical Sciences (Kunming, China) were incubated in RPMI-1640 medium (Gibco-BRL, Carlsbad, USA) supplemented with 10% fetal bovine serum (complete medium) at 37°C with 5% CO_2. Until the cells were cultured to ~70% confluence, the recombination over-expression or knockdown plasmids of *MTERF1* were transfected into HeLa cells using Lipofectamine 2000 (Invitrogen) with the designed constructs according to the manufacturer's protocol.

Semi-quantitative reverse transcription-PCR

Total RNA extraction and cDNA synthesis from HeLa cells were done as mentioned above. The *MTERF1* mRNA expression levels of HeLa cells transfected with plasmids MTERF1-Flag, pSi-MTERF1, and empty vectors were detected by reverse transcription-PCR (RT-PCR), respectively. Four heavy-chain (H) genes (*12S rRNA*, *16S rRNA*, *ND1*, and *COXI*) and one light-chain (L) gene (*ND6*) were selected to represent mitochondrial genes, and then their mRNA levels were qualified by semi-quantitative RT-PCR. The mRNA expression levels of *β-actin* were also examined (primers for PCR detection are listed in **Table 1**). PCR was done as follows: 1 cycle at 94°C for 3 min, 26 cycles at 94°C for 30 s, 58°C for 30 s, and then 72°C for 1 min, followed by 1 cycle at 72°C for 10 min. The resulting PCR products were isolated by agarose gel electrophoresis, after which the DNA band intensities were quantified using a Gel Doc1000 Gel Scanner (Bio-Rad, Hercules, USA).

mtDNA replication assay

DNA was isolated from HeLa cells using a TIANamp Genomic DNA Extraction Kit (Tiangen, Beijing, China) according to the manufacturer's protocols. The expression level of *D-loop* of mitochondria was detected to represent the mtDNA replication level and *18S rRNA* as an internal reference. Primers for PCR detection of D-loop DNA replication were as follows: 5′-GGGAACGTGTGGGCTATTTA-3′ (forward), 5′-TACTCAAATGGGCCTGTCCT-3′ (reverse). The primers for human *18S rRNA* were: 5′-CGCGCTCTA CCTTACCTACC-3′ (forward) and 5′-CCGTCGGCATGTA TTAGCTC-3′ (reverse). PCR was performed as follows:

Table 1. Primers for semi-quantitative RT-PCR

Gene	Primer sequence	
MTERF1	Sense:	5′-CCATTATGGCACCAGGAAACC-3′
	Anti-sense:	5′-CTCCACAGATCCCACCGTTTT-3′
12S rRNA	Sense:	5′-AAACTGCTCGCCAGAACACT-3′
	Anti-sense:	5′-CATGGGCTACACCTTGACCT-3′
16S rRNA	Sense:	5′-CACTGTCAACCCAACACAGG-3′
	Anti-sense:	5′-GGCAGGTCAATTTCACTGGT-3′
ND1	Sense:	5′-TGATCAGGGTGAGCATCAAA-3′
	Anti-sense:	5′-GGTTCGGTTGGTCTCTGCTA-3′
COXI	Sense:	5′-GATTTTTCGGTCACCCTGAA-3′
	Anti-sense:	5′-CGGAGGTGAAATATGCTCGT-3′
ND6	Sense:	5′-CCACAGCACCAATCCTACCT-3′
	Anti-sense:	5′-TGATTGTTAGCGGTGTGGTC-3′
β-actin	Sense:	5′-CAGGAAGGAAGGCTGGAAG-3′
	Anti-sense:	5′-CGGGAAATCGTGCGTGAC-3′

1 cycle at 94°C for 3 min, 22 cycles at 94°C for 30 s, 58°C for 30 s, and then 72°C for 1 min, followed by 1 cycle at 72°C for 10 min. The resulting PCR products were isolated by agarose gel electrophoresis, and the DNA band intensities were quantified by the Gel Doc1000 Gel Scanner.

Western blots analysis

Western blot analysis was performed as previously described [18]. The protein was extracted using cell lysis buffer (Beyotime, Shanghai, China), lyzed on ice for 30 min, and centrifuged at 15 000 *g* for 10 min at 4°C. Protein concentrations were measured by BCA reagents (Dingguo Biotechnology, Beijing, China) with bovine serum albumin (BSA) as a standard. The supernatant was mixed with 5× sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) loading buffer (CWBIO, Beijing, China), boiled for 5 min, and separated by 8% SDS–PAGE electrophoresis using standard methodology. The proteins in the gel were transferred to the polyvinylidene difluoride membrane (Millipore, Bedford, USA) with wet transfer electrophoresis (Bio-Rad) for 90 min at 100 V. The membranes was blocked with 5% fat-free milk in phosphate buffer saline (PBS) for 2 h at room temperature, and then incubated with the primary antibodies against ND1, ND6, COX1, cyclin D1, or β-actin overnight at 4°C with gentle shaking. After three times wash, the membranes were probed with suitable secondary antibodies coupled with horseradish peroxidase (Santa Cruz, Santa Cruz, USA) for 1 h at room temperature. Signals were detected with an enhanced chemiluminescence kit (Bio-Rad). Antibodies against COX I, ND6, ND1, and cyclin D1 were purchased from Santa Cruz Biotechnology (Santa Cruz, USA), and anti-β-actin antibody was obtained from Anbo Biotechnology Company (San Francisco, USA). Anti-MTERF1 antibody was purchased from Abcam Biotechnology Company (Cambridge, UK).

ATP determination

ATP was extracted from cells and its concentration was measured using an ATP Assay Kit (Beyotime). Protein concentrations were estimated by Bradford's method using BSA [19].

MTT cell proliferation assay

HeLa cells were seeded in 24-well plates at a density of 1×10^5 cells per well with the complete M1640 medium, and then incubated at 37°C with 5% CO_2. Cells were transfected with MTERF1-Flag, pSi-MTERF1, or empty vector. After 0, 24, and 48 h of culture, each well was added 20 μl of 5 mg/ml 3-(4,5)-dimethylthiahiazo(-2-y1)-3,5-diphenytetrazolium bromide (MTT) solution. Cells were then incubated for another 4 h while they absorbed the nutrient solution. Dimethyl sulfoxide (150 μl) was then added into wells, and the plates were shaken for 10 min to dissolve formazan crystals. The absorbance at 570 nm was then read by a microplate reader (Bio-Rad). The cell numbers in each well were verified via optical density. All experimental procedures were done in triplicate.

Flow cytometry assay

HeLa cells were transfected with MTERF-Flag, pSi-MTERF1, and their control plasmids. After 48 h, cells were trypsinized and harvested by centrifugation at 300 *g* for 5 min. Cells were then resuspended in cold PBS, fixed with 70% ethanol for 1 h at 4°C, and then washed twice with cold PBS and resuspended in 1 ml PBS, followed by staining with 100 μl propidium iodide (PI) at 4°C for 30 min (final concentration of PI was 100 μg/ml). Following this process, cells were analyzed for

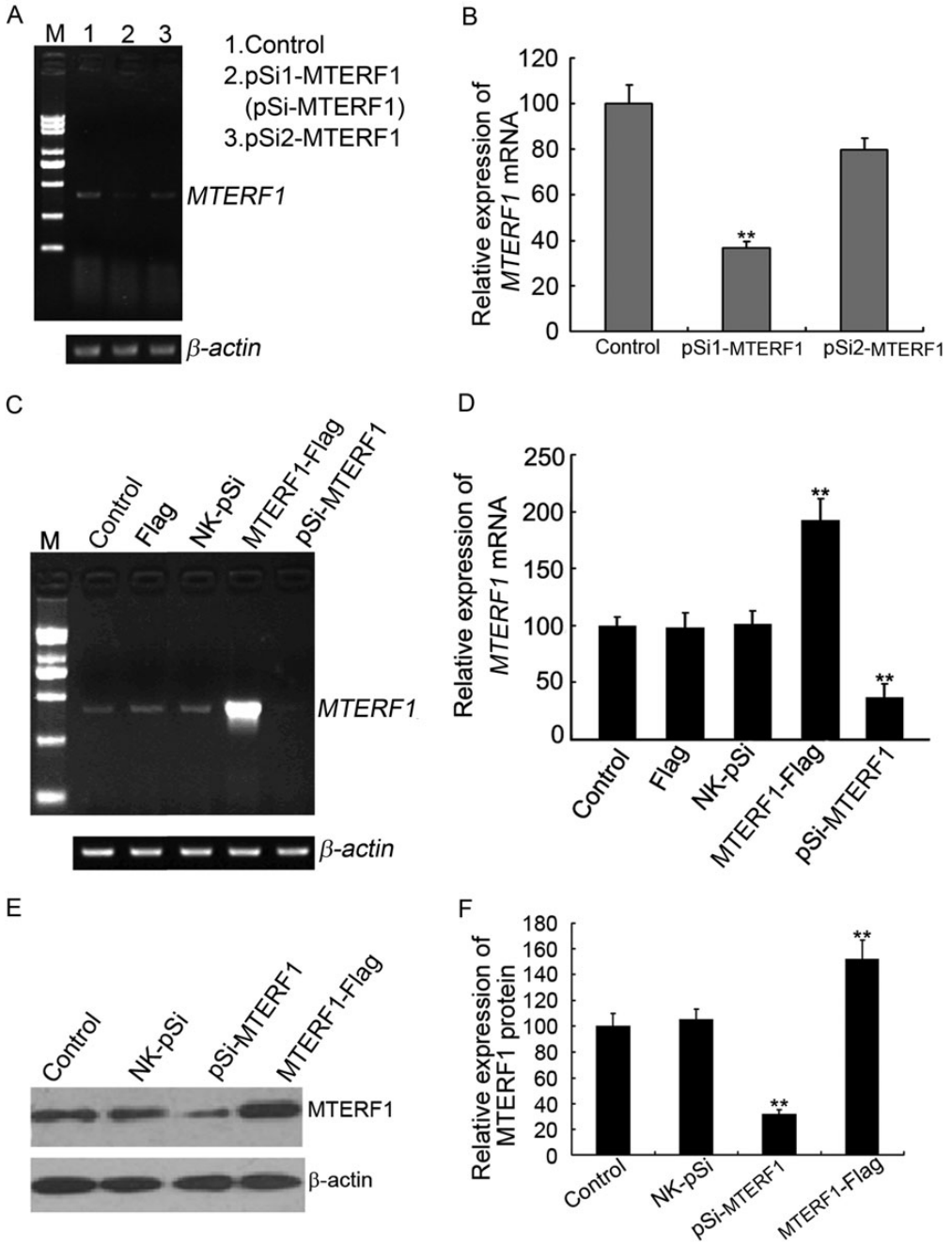

Figure 1. The expression of MTERF1 in the transfected cells MTERF1-Flag, pSi1-MTERF1, pSi2-MTERF1, and vector control were transfected into HeLa cells, respectively. Cells were harvested at 48 h after transfection, the total RNA was extracted and subject to RT-PCR analysis. The MTERF1 protein expression was analyzed by western blotting. (A) The mRNA levels of *MTERF1* in the pSi1-MTERF1, pSi2-MTERF1, and control vector transfected cells. (B) The quantification of the mRNA expression levels of *MTERF1* relative to *β-actin* gene. (C) The electrophoresis picture of the mRNA levels of *MTERF1*. (D) The quantification of the mRNA expression levels of *MTERF1* relative to *β-actin* gene. (E) The western blot picture of MTERF1 (top), and β-actin (bottom). (F) The quantification of the MTERF1 protein expression levels relative to β-actin. $^{**}P < 0.01$, significant differences compared with the control.

cell cycle progression using a fluorescence activated cell sorter (Becton-Dickinson, Franklin Lakes, USA).

Statistical analysis

All data were expressed as mean ± standard error of mean. Statistical analysis was performed by two-tailed unpaired Student's *t*-test. $P < 0.05$ was considered statistically significant and $P < 0.01$ was regarded as highly significant. All the experiments in this study were done in triplicate.

Results

The expression of MTERF1 in the transfected cells

To confirm the transfection effect of recombinant plasmids MTERF1-Flag, pSi1-MTERF1, and pSi2-MTERF1,

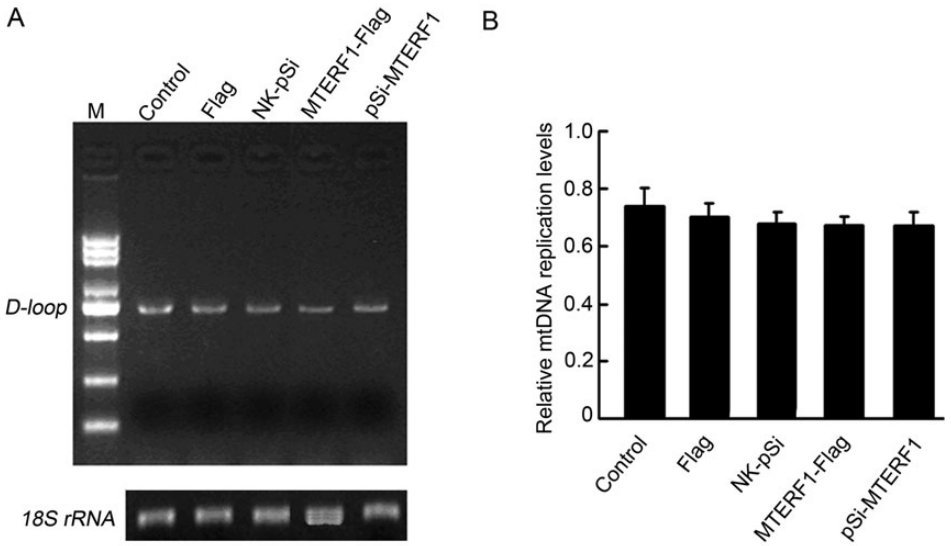

Figure 2. MTERF1 has no significant effect on the replication of human mtDNA MTERF1-Flag, pSi1-MTERF1, and vector control were transfected into HeLa cells, respectively. Cells were harvested at 48 h after transfection, the total RNA was extracted and subject to RT-PCR analysis. (A) The electrophoresis picture of *D-loop* replication levels. (B) The quantification of *D-loop* replication levels relative to *18S rRNA* gene.

semi-quantitative RT-PCR and western blot analysis were used to detect the expression of MTERF1 in the transfected cells. Semi-quantitative RT-PCR results showed that compared with control, pSi1-MTERF1 could inhibit about 64% of the expression of *MTERF1* and pSi2-MTERF1 inhibited 21% of the expression of *MTERF1* (**Fig. 1A,B**). So, we chose pSi1-MTERF1 (named as pSi-MTERF1) as an optimal interference plasmid to carry out the subsequent experiments. In the MTERF1-Flag transfected cells, the mRNA expression of *MTERF1* was increased to 1.92 times of the control. In contrast, the *MTERF1* mRNA level in the cells transfected with pSi-MTERF1 decreased to 36% of the control, indicating that the transcription of endogenous genes was dramatically inhibited (**Fig. 1C,D**).

Likewise, the MTERF1 protein level in the cells transfected with MTERF1-Flag increased to 1.52 times of the control. However, in the cells transfected with pSi-MTERF1, the MTERF1 protein level decreased to 32% of the control (**Fig. 1E,F**). All the data indicated that the over-expression and knockdown vectors of MTERF1 can significantly regulate the expression of MTERF1 in the transfected HeLa cell.

MTERF1 has no significant effect on the replication of human mtDNA

To study whether MTERF1 regulated the replication of human mtDNA, recombinant plasmids of MTERF1-Flag and pSi-MTERF1 were transfected into HeLa cells. After 48 h, total DNA was extracted to serve as a template for PCR (**Fig. 2A**). The mitochondrial *D-loop* region was amplified and normalized by housekeeping gene *18S rRNA*. Results showed that the MTERF1 had no significant effect on the replication of human mtDNA (**Fig. 2B**).

MTERF1 positively regulates mitochondrial genes transcription

To determine the effect of MTERF1 on mtDNA transcription, we selected five representative mitochondrial genes: the H chain of *12S rRNA*, *16S rRNA*, *ND1*, and *COXI*, and the L chain of *ND6*. Semi-quantitative RT-PCR results showed that over-expression of *MTERF1* was able to significantly up-regulate the transcription levels of *12S rRNA*, *16S rRNA*, and *ND1* (**Fig. 3A,B**). In contrast, the knockdown of *MTERF1* decreased the expression levels of all these mitochondrial genes. Together, these results indicated that MTERF1 is a positive transcriptional regulatory factor (**Fig. 3C,D**).

Likewise, we performed western blot to detect the expression levels of two proteins in the heavy chain (ND1, COXI) and one in the light chain (ND6) of mitochondria. The results showed that among the over-expressed MTERF1 samples, the levels of all the three mitochondrial proteins were not significantly increased compared with those in the control cells. However, the knockdown of MTERF1 resulted in decreased levels of all these mitochondrial proteins. The fold changes in the protein levels were similar to those of mitochondrial mRNA transcription (**Fig. 4A,B**). Collectively, these findings indicated that mitochondrial transcription regulation is more complex than previously anticipated.

MTERF1 is a positive regulation for cellular ATP concentration

To study the effects of MTERF1 on OXPHOS, we detected the ATP concentrations in the cells transfected with MTERF1-Flag, pSi-MTERF1, or the empty vector. Our results indicated that MTERF1 is a positive regulator of cellular ATP concentration. Over-expression of *MTERF1*

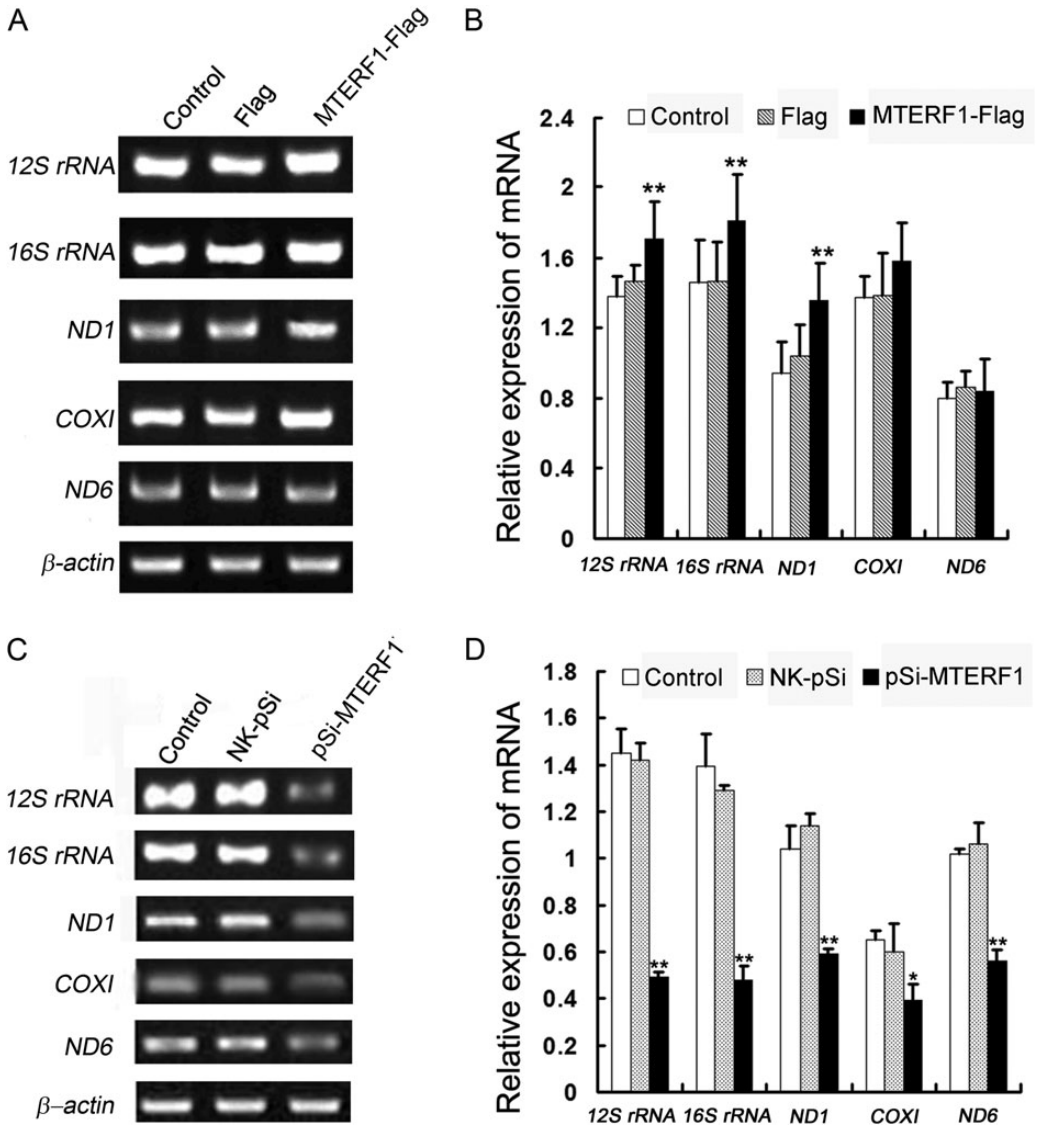

Figure 3. MTERF1 positively regulates the expression levels of mitochondrial mRNAs HeLa cells were transfected with MTERF1-Flag, pSi-MTERF1, and the empty vector. (A) Gel images of mitochondrial mRNAs' expression levels in *MTERF1* over-expressed cells. *β-actin* was employed as a loading control. (B) Relative levels of mitochondrial mRNAs in *MTERF1* over-expressed cells. (C) The electrophoresis picture of mitochondrial mRNAs' expression levels in *MTERF1* knockdown cells. *β-actin* was employed as the loading control. (D) Relative levels of mitochondrial mRNAs in *MTERF1* down-regulated cells. $^{**}P < 0.01$, highly significant differences compared with the control. $^{*}P < 0.05$, significant differences compared with the control.

caused a slight increase of ATP production, but knockdown of *MTERF1* resulted in a significant decrease compared with the control cells (**Fig. 5**).

MTERF1 can promote the proliferation of HeLa cells

To investigate whether MTERF1 is able to promote the growth of cells, the cell proliferation was examined at 0, 24, and 48 h post-transfection by MTT assay. Compared with the control, the number of viable cells transfected with MTERF1-Flag increased at 24 h post-transfection with a significant difference at 48 h. In contrast, the number of viable cells transfected with pSi-MTERF1 was markedly reduced at 24 h and remained lower at 48 h (**Fig. 6**). These results indicated that MTERF1 may be important for cell proliferation, because knockdown of this gene inhibited cell growth while over-expression promoted cell growth.

To further confirm the role of MTERF1 in regulating cell proliferation, we investigated the cell cycle of those cells transfected with MTERF1-Flag, pSi-MTERF1, or the empty vector. Flow cytometric analysis showed that *MTERF1* knockdown-induced cell growth inhibition was due to the dysregulation of cell cycle. Compared with the control, cells transfected with the pSi-MTERF1 displayed an increase in the percentage of cells at the G0/G1 phase (78.28% ± 2.3%), and a decrease in the S phase (4.1% ± 0.9%) and in the G2/M phase (10.2% ± 1.7%). In contrast, among cells transfected with MTERF1-Flag, the percentage of G0/G1 cells was lower, whereas the percentage of S and G2/M

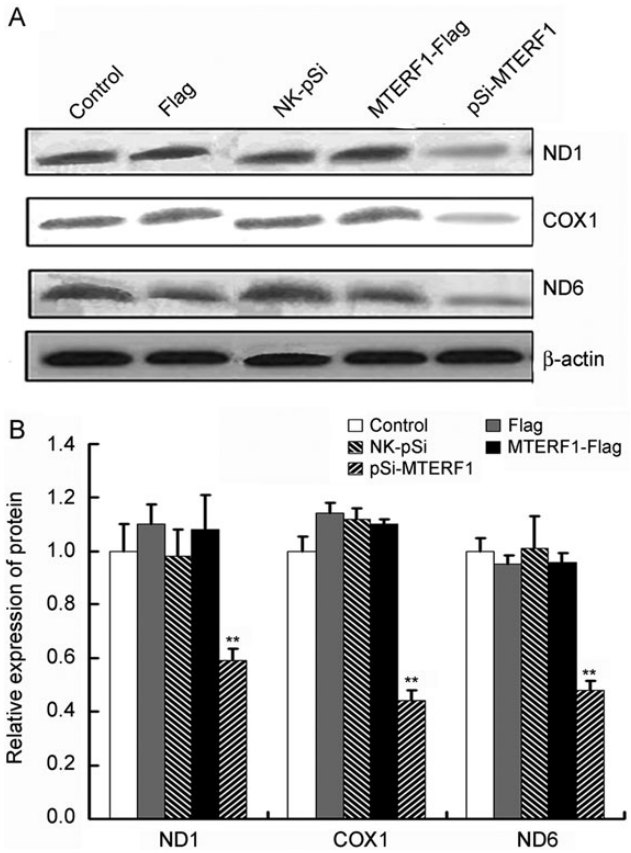

Figure 4. Knockdown of *MTERF1* decreases the translation levels of mitochondrial proteins HeLa cells were transfected with MTERF1-Flag, pSi-MTERF1, and vector control. (A) The western blot picture of ND1 (top), COXI, ND6 (middle), and β-actin (bottom). (B) The quantification of the ND1, COXI, and ND6 protein expression levels relative to β-actin. $^{**}P < 0.01$, highly significant differences compared with the control.

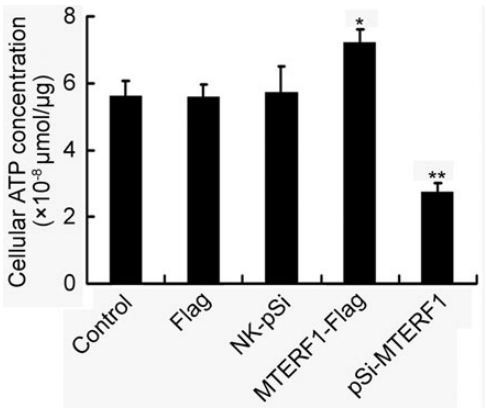

Figure 5. The effect of *MTERF1* expression on cellular ATP levels HeLa cells were transfected with MTERF1-Flag, pSi-MTERF1, and vector control plasmids. $^{**}P < 0.01$, highly significant differences compared with the control group. $^{*}P < 0.05$, significant differences compared with the corresponding control group.

cells was higher than those cells in the control. Together, these results suggested that over-expression of *MTERF1* may promote cell proliferation, while down-regulation of

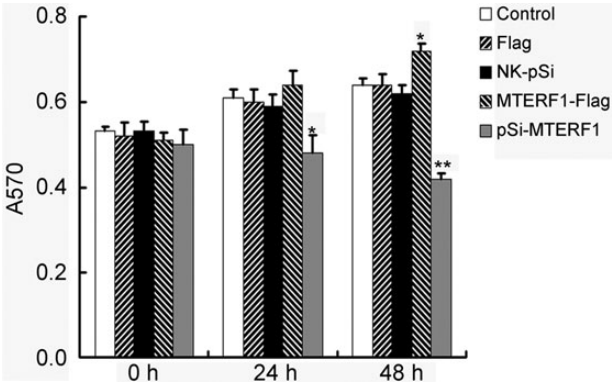

Figure 6. MTERF1 promotes the proliferation of HeLa cells HeLa cells were transfected with MTERF1-Flag, pSi-MTERF1, and empty vector, respectively. Cells were treated with MTT for 4 h at 0, 24, and 48 h post-transfection. MTT assay was performed in triplicate. $^{**}P < 0.01$, highly significant differences compared with the corresponding control. $^{*}P < 0.05$, significant differences compared with the corresponding control.

MTERF1 leads to cell accumulation in G0/G1 phase and cell growth inhibition (**Fig. 7A,B**).

Considering that cyclin D1 protein is a key protein that makes cells traverse the G1 phase and enter S phase [20], we studied whether MTERF1 can regulate the expression of cyclin D1. The results demonstrated that the level of cyclin D1 protein in the cells transfected with MTERF1-Flag was higher than that in the control cells. On the contrary, the expression levels of cyclin D1 protein was significantly lower in the cells transfected with pSi-MTERF1 compared with that in the control cells (**Fig. 8A,B**). These data suggested that MTERF1 can also regulate the expression of cyclin D1 in HeLa cells.

Discussion

Mitochondria are often known as the power plants of eukaryotic cells, as they are responsible for oxidizing raw materials and storing the resulting energy at ATP. There are numerous multi-enzyme complex proteins located in the intima of mitochondria that form the foundation of the electron transport system known as the respiratory chain. OXPHOS is associated with the oxidation process of the respiratory chain which then generates ATP [21]. The mitochondrial OXPHOS system, which produces the main source of cellular ATP that is needed for cell growth, consists of 5 complex proteins encoded by nuclear genes and 13 mitochondrial genes. ATP synthesis is co-controlled by the proteins encoded by both these nuclear genes and mitochondrial genes [22–24]. Previous research has shown that mitochondrial OXPHOS process and cellular ATP are critical for cell proliferation [25–30]. Likewise, cyclin D1 is an important factor regulating cellular G1 phase, and reaches its highest levels during the

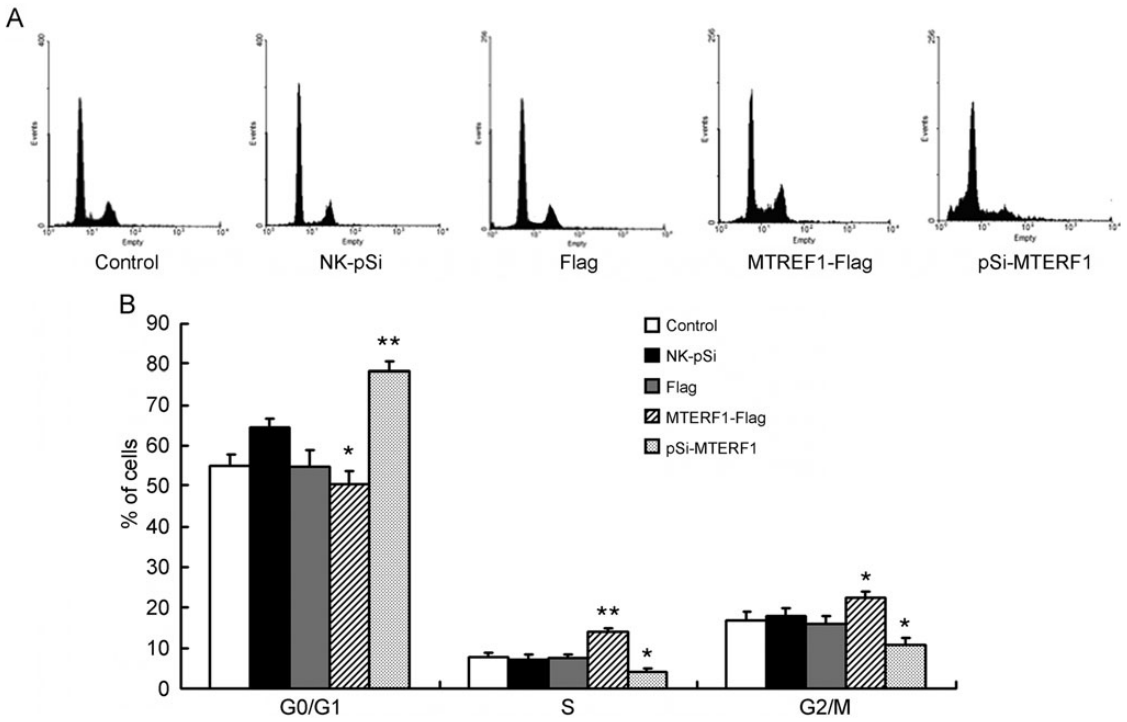

Figure 7. Knockdown of MTERF1 leads to HeLa cells arrest in G0/G1 phase (A) Representative histograms of flow cytometry analysis showed the cell cycle of HeLa cells transfected with MTERF1-Flag, pSi-MTERF1, or empty vector control. (B) Distribution in different cell cycle phases among cells transfected with MTERF1-Flag, pSi-MTERF1, or the control vectors. $^{**}P < 0.01$, highly significant differences compared with the control. $^{*}P < 0.05$, significant differences compared with the control.

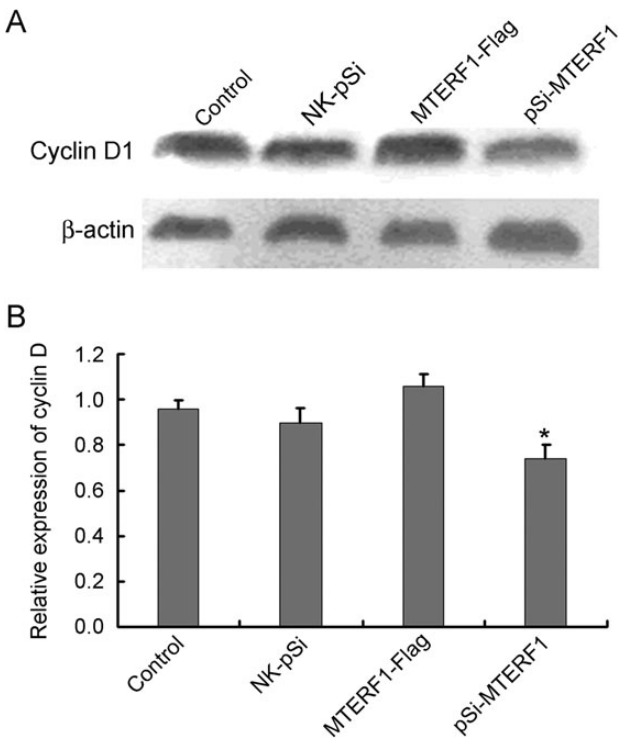

Figure 8. Knockdown of *MTERF1* decreases the expression level of cyclin D1 protein HeLa cells were transfected with MTERF1-Flag, pSi-MTERF11, and empty vector control. (A) The western blot picture of cyclin D1 (top), and β-actin (bottom). (B) The quantification of the cyclin D1 protein expression levels relative to β-actin. $^{*}P < 0.05$, significant differences compared with the control.

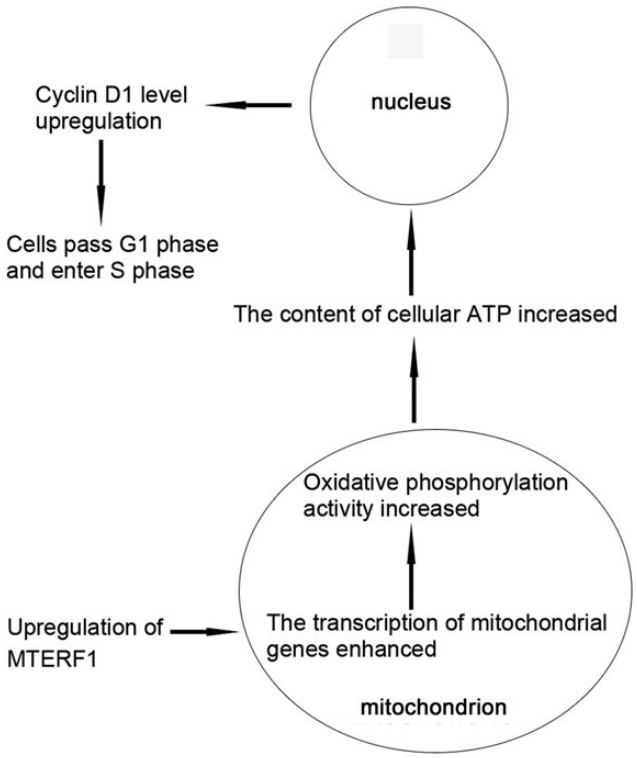

Figure 9. Potential mechanism of *MTERF1* on regulating cell proliferation

mid-G1 phase; cyclin D1 is so important that lower levels of it will actually arrest the cell cycle at this phase [31,32]. Gemin *et al*. [33] noted that oligomycin-treated HL-60 cells both reduced the levels of ATP and cyclin D1 levels. There are two sides of ATP's effect on cell growth. On one hand, ATP acts as an intracellular intermediate for the transfer of energy, so when it is higher, cell growth is better [5]. On the other hand, ATP acts as a signaling molecule, and when ATP concentration reaches a certain threshold, cells are activated and express cyclin D1, at which point they are prompted cells to pass the G1 phase [33].

Recent studies of mitochondrial transcription termination factors mainly focused on the manner of mtDNA binding and transcriptional regulation of mitochondrial genes, almost completely ignoring the link between the regulation of MTERF1 and the mitochondrial gene transcription and cell proliferation regulation. In this study, we discovered that the expression of MTERF1, mitochondrial gene expression, and cellular ATP levels are connected with cell proliferation progression. Our results showed that the MTERF1 played no significantly observable role in the replication of human mtDNA, but that MTERF1 is a positive regulatory factor for the transcription mitochondrial genes. Over-expression of MTERF1 enhanced the transcription of mitochondrial genes, while conversely down-regulation of MTERF1 weakened the transcription of mtDNA. Over-expression of MTERF1 can also increase the ATP production and the expression of cyclin D1, and promote cell proliferation in HeLa cells. However, down-regulation of MTERF1 decreases cellular ATP production and the expression of the cyclin D1 protein. Interestingly, down-regulation of MTERF1 also appeared to inhibit cell proliferation, resulting in cell cycle arrest at G0/G1 phase.

These collective results suggest that MTERF1 may affect oxidative phosphorylation activity by regulating the transcription of oxidative phosphorylation complex subunits encoded by genes on the mitochondrial genome. Similarly, these results suggest that MTERF1 is the one of necessary factors for maintaining normal cell growth, being associated with the cell energy supply and promoting cell proliferation. This may point to an as-of-yet unknown mechanism(s) by which MTERF1 promotes cell proliferation (**Fig. 9**). When MTERF1 is up-regulated, it enhances the transcription of oxidative phosphorylation subunits (*ND1*, *COXI*, *ND6*, and so on) which are encoded by genes on the mitochondrial genome. In doing so, oxidative phosphorylation activity increases, and accordingly, cellular ATP concentrations increases as well, enhancing the expression of the cyclin D1 protein and pushing the cells past the G1 phase and into the S phase.

Our results are exciting novel findings on how MTERF1 is involved in the regulations of oxidative phosphorylation and cell proliferation in HeLa cells. These results provide evidence that MTERF1 plays an important role in cell growth.

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