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Original Article

Mitochondrial transcription termination factor 2 binds to entire mitochondrial DNA and negatively regulates mitochondrial gene expression

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Mitochondrial transcription termination factor 2 (mTERF2) is a mitochondrial matrix protein that binds to the mitochondrial DNA. Previous studies have shown that overexpression of mTERF2 can inhibit cell proliferation, but the mechanism has not been well defined so far. This study aimed to present the binding pattern of mTERF2 to the mitochondrial DNA (mtDNA) *in vivo*, and investigated the biological function of mTERF2 on the replication of mtDNA, mRNA transcription, and protein translation. The mTERF2 binding to entire mtDNA was identified via the chromatin immunoprecipitation analysis. The mtDNA replication efficiency and expression levels of mitochondria genes were significantly inhibited when the mTERF2 was overexpressed in HeLa cells. The inhibition level of mtDNA content was the same with the decreased levels of mRNA and mitochondrial protein expression. Overall, the mTERF2 might be a cell growth inhibitor based on its negative effect on mtDNA replication, which eventually down-regulated all of the oxidative phosphorylation components in the mitochondria that were essential for the cell's energy metabolism.

Keywords mTERF2; cell proliferation; chromatin immunoprecipitation; mitochondrial DNA

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Introduction

Oxidative phosphorylation (OXPHOS) is a highly coordinated cellular process that is regulated by both nuclear genome and mitochondrial genome. Mitochondrial DNA (mtDNA) only encodes proteins of the OXPHOS complexes but not any regulatory factors for the mitochondria gene expression [1–4]. The nuclear genome encodes mitochondrial regulatory proteins that enter the mitochondria and regulate mitochondrial activities directly or indirectly through regulating the expression of mitochondrial genes. Recently, several mitochondrial regulators encoded by nuclear genome have been identified [5,6]. For example, Yu *et al*. [7] have reported that the mitochondrial transcription termination factor 4 (*hMTERF4*) knockdown in HeLa cells results in sub-G1 cell accumulation and cell death, which suggested that hMTERF4 may be probably modulated by mitochondrial transcription to promote cell proliferation. But how can they regulate mitochondrial gene expression *in vivo* remains unclear.

mTERF2 belongs to mitochondrial transcription termination factor family, which shares 29% amino acid identity and 52% protein similarity with mTERF1 [8]. mTERF2 is a mitochondrial matrix protein associated with the inner membrane [9], but its binding pattern on the mtDNA is still a controversy. Evidence supports both the non-specific mtDNA-binding [10,11] and the specific binding to the heavy strand promoter (HSP) site when functioning as a positive regulator of transcription [12]. mTERF2 protein is localized in mitochondrial matrix [13] and binds to the mtDNA [10–12], which suggests a role of mTERF2 in regulating the mtDNA replication or mitochondrial gene transcription.

Hyvarinen *et al*. [11] have reported that overexpression of mTERF2 results in the depletion of mtDNA copy numbers and an accumulation of specific fragment of mtDNA replication. Some researches suggested that the mTERF2 would promote the mitochondrial gene transcription [10], similar to the function of mTERF1 [8,14–19]. Based on these contradictory results, it is necessary to further explore the effects of mTERF2 on mtDNA replication, transcription, and translation.

In order to identify the binding pattern of mTERF2 and study the significance of the binding activity on the entire

mtDNA, the mTERF2 binding sites on the mtDNA were screened using chromatin immunoprecipitation (ChIP) analysis, and the function of mTERF2 on mtDNA replication and mitochondria gene expression were also analyzed.

Materials and Methods

Cell culture, plasmids, and transfection

HeLa cells were kindly provided by Dr Guoyang Liao (Institute of Medical Biology, Chinese Academy of Medical Sciences, Kunming, China), and were maintained in RPMI 1640 medium (Gibco-BRL, Gaithersburg, USA) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. The open reading frame of *mTERF2* was cloned from HeLa cells in our previous study and inserted into a eukaryotic expression vector pcDNA3.1(+) (Invitrogen, Carlsbad, USA). The recombinant plasmid pcDNA-mTERF2 and the control empty plasmid pcDNA3.1(+) were transfected separately into HeLa cells with Lipofectamine 2000 (Invitrogen) reagent according to the manufacturer's instructions. The transfected cells were used for the determination of DNA content, mRNA, and protein levels.

ChIP analysis

Cells were cultured in a 10-cm dish to obtain as least 10^6 cells. Formaldehyde was added directly to culture medium to a final concentration of 1%, and incubated for 10 min at 37°C to cross-link the DNA–protein complex. Then the medium was removed. Cells were washed twice with cold phosphate-buffered saline (PBS) containing protease inhibitors (1 mM phenylmethanesulfonyl fluoride, 1 μg/ml aprotinin and 1 μg/ml pepstatin A), and scraped into a micro centrifuge tube. Then cells were harvested by spin at 2000 *g* at 4°C for 4 min, resuspended in 200 μl sodium dodecyl sulfate (SDS) lysis buffer and placed on the ice for 10 min. The genome DNA was sheared with deoxyribonuclease I (DNase I) at 37°C for 1 h, and 0.5 M ethylenediaminetetraacetic aid (EDTA) was added to terminate the reaction. Samples were centrifuged (13,000 *g*) for 10 min at 4°C, and the supernatant was transferred to a fresh tube. An aliquot of the supernatant was used to extract DNA, and the DNA digestion length was detected by 1% agarose gel. The DNA fragments of around 1 kb were employed as the input control.

The supernatant obtained above was diluted to 2 ml by ChIP dilution buffer (containing protease inhibitors). In order to reduce non-specific background, 2 ml diluted cell supernatant was pre-cleared with 75 μl salmon sperm DNA/protein A agarose-50% slurry and slurred for 30 min at 4°C with agitation. After a brief centrifugation, supernatant was collected into a new tube, anti-mTERF2 antibody (1:50 dilution) (Abcam, Cambridge, UK) was added and the tube was incubated with rotation at 4°C overnight. For negative antibody control, equal amount of anti-arrest-defective protein 1 antibody (1:50 dilution) was added in an aliquot of the supernatant, and sample was processed similarly in parallel. On the second day, 60 μl of salmon sperm DNA/protein A agarose-50% slurry was added into tubes. After rotation for another 2 h at 4°C, the tubes were centrifuged (1000 *g* at 4°C for 1 min), the supernatant containing unbound, non-specific DNA was discarded, and then the pellet was washed for 5 min on a rotating platform with 1 ml each of the following buffers in the order as listed: (i) low-salt immune complex wash buffer, once; (ii) high-salt immune complex wash buffer, once; (iii) LiCl immune complex wash buffer, once; (iv) TE buffer, twice. After the final wash and centrifugation, 250 μl elution buffer (1% SDS and 0.1 M $NaHCO_3$) was added to the pellet, mixed by a brief vortex, and incubated at room temperature for 15 min with rotation. Agarose beads were spun down, and the supernatant containing eluted mTERF2 was transferred carefully to another tube. The elution was repeated two times, and all the eluent was combined into a new tube.

About 20 μl of NaCl (5 M) was added into the tube, and the mixture was heated at 65°C for 4 h to release the DNA bound to mTERF2. Then proteins were digested with 2 μl of 10 mg/ml proteinase K (Merck, San Diego, USA) in 10 μl of 0.5 M EDTA and 20 μl of 1 M Tris-HCl (pH 6.5) for 3 h at 45°C. DNA was extracted with standard phenol/chloroform method and precipitated by ethanol. DNA pellets were washed with 70% ethanol, dried in the air, and resuspended in 100 μl ddH_2O for polymerase chain reaction (PCR). Seventeen primer pairs covering the entire human mitochondrial genome were used for PCR (**Table 1**). The average length of amplified products was approximately 1 kb. PCR products were visualized on 1% agarose gel, and quantified using Quantity One 4.6 software (Bio-Rad, Hercules, USA).

Determination of mtDNA level

At 36 h after transfection with pcDNA-mTERF2 or control vector, the cells were collected from the culture dish with a cell scraper. Cells were washed twice with 1 × PBS, and then resuspended in cell lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, and 1 mM Na_3VO_4). Then cells were lyzed for 10 min on the ice, and cell lysate was treated with proteinase K (10 mg/ml) and 10% SDS overnight at 45°C. Then, the sample was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1). DNA was then precipitated with 1/10 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of 100% cold ethanol. After centrifugation at 10,000 *g* for 5 min at 4°C, the liquid was removed. DNA pellet was

Table 1 The primers used in ChIP analysis

No.	Primer sequence (5′→3′)	Site	Product length (bp)
1	F: GTATGCACGCGATAGCATTG		
	R: TAGGCTGAGCAAGAGGTGGT	71－1256	1186
2	F: CGATCAACCTCACCACCTCT		
	R: CCAATTGGGTGTGAGGAGTT	1226－2269	1044
3	F: AAGCGTTCAAGCTCAACACC		
	R: TGGGTGGGTGTGGGTATAAT	2199－3217	1019
4	F: CCTCCCTGTACGAAAGGACA		
	R: GGGGAATGCTGGAGATTGTA	3116－4252	1137
5	F: GATTCCGCTACGACCAACTC		
	R: TGATGGTGGCTATGATGGTG	4142－5325	1184
6	F: ATCATCCCCACCATCATAGC		
	R: TGAAATTGATGGCCCCTAAG	5297－6396	1100
7	F: TGGAGCCTCCGTAGACCTAA		
	R: AGCGAAGGCTTCTCAAATCA	6320－7334	1015
8	F: GAACCCTCCATAAACCTGGA		
	R: TATGGTGGGCCATACGGTAG	7361－8401	1041
9	F: ATGGCCCACCATAATTACCC		
	R: GTGGCCTTGGTATGTGCTTT	8388－9416	1029
10	F: TTCCACTCCATAACGCTCCT		
	R: GTGGCAGGTTAGTTGTTTGT	9315－10314	1000
11	F: CCCTACCATGAGCCCTACAA		
	R: TTCTTGGGCAGTGAGAGTGA	10278－11314	1037
12	F: TGAACGCAGGCACATACTTC		
	R: AGGGGTCGTAAGCCTCTGTT	11187－12198	1012
13	F: ACACCTATCCCCCATTCTCC		
	R: GGTGGAAGCGGATGAGTAAG	12076－13129	1054
14	F: GCCCTACTCCACTCAAGCAC		
	R: TATGCCTTTTTGGGTTGAGG	13079－14085	1007
15	F: CCTCAACCCAAAAAGGCATA		
	R: ATGCCGATGTTTCAGGTTTC	14066－15096	1031
16	F: TATCCGCCATCCCATACATT		
	R: AGCTTTGGGTGCTAATGGTG	15195－15997	803
17	F: TACTCAAATGGGCCTGTCCT		
	R: GGGAACGTGTGGGCTATTTA	15873－16549	676

washed twice with 70% ethanol, air-dried, and then dissolved into pure H_2O, using as the PCR template.

The mtDNA level was assessed using methods described previously [20,21]. D-Loop region of mtDNA and 18S ribosomal RNA (rRNA) coding gene were amplified with the extracted template, and the expression ratio of mtDNA D-Loop region to 18S rRNA was determined as the relative level of mtDNA in cells. The sequences of primer pairs for the mtDNA D-Loop region were 5′-TACTCAAATGGGC CTGTCCT-3′ and 5′-GGGAACGTGTGGGCTATTTA-3′; and those for 18S rRNA coding gene were 5′-AGAGGGA CAAGTGGCGTTCAGC-3′ and 5′-GCACACCAACGACA CGCCCTT-3′. PCR was carried out in exactly the same way as the steps in the reverse transcriptase–PCR (RT–PCR) procedure described below.

Semi-quantitative RT－PCR to detect the mitochondrial RNA level

In order to determine the mRNA transcription levels of mitochondrial genes, seven genes covering both the heavy and light chains of mtDNA were quantified by RT－PCR with primer pairs shown in **Table 2**.

Cells transfected with pcDNA-mTERF2 or pcDNA3.1(+) plasmids were rinsed with cold PBS once, and then lyzed directly in a culture dish with Trizol reagent (Invitrogen). Total RNA was extracted according to the

Table 2 The primers used in semi-quantity RT-PCR

Mitochondrial gene	Primer sequence (5′→3′)
12S rRNA	F: ACTGCTCGCCAGAACACT
	R: TGGGCTACACCTTGACCT
16S rRNA	F: CTGTCAACCCAACACAGG
	R: CAGGTCAATTTCACTGGT
ND1	F: ATCAGGGTGAGCATCAAA
	R: TTCGGTTGGTCTCTGCTA
COXII	F: ATCCCTACGCATCCTTTA
	R: GGAATTGCATCTGTTTTT
Cytb	F: TCCGCCATCCCATACATT
	R: GGGCGTCTTTGATTGTGT
ND6	F: ACAGCACCAATCCTACCT
	R: ATTGTTAGCGGTGTGGTC
Q tRNA	F: ATCCCTGAGAATCCAAAA
	R: GGGCCCGATAGCTTATTT
β-actin	F: ATCCCTGAGAATCCAAAA
	R: GGGCCCGATAGCTTATTT

manufacturer's protocol. About 5 μg total RNA was adjusted to 36 μl with RNase free pure water, mixed with 1 μl of 6-bp random primers (100 M) and 15.1 μl oligo (dT). The RT reaction tube was incubated at 70°C for 5 min, and then placed on ice. The reaction mixture included 12 μl of 5×RT-buffer (Promega, Madison, USA), 4 μl of 10 mM deoxynucleoside triphosphate , 2 μl of RNasin, and 1 μl of M-MLV reverse transcriptase (Promega). The mixture was incubated at 25°C for 5 min, then at 37°C for 2 h, and terminated at 70°C for 10 min. The cDNA was kept on the ice.

The cDNA sample was used for PCR with primers specific for mitochondrial genes or the *β-actin* gene (a control for sample input). PCR reactions were carried out under the following steps: denaturation at 94°C for 3 min; 25 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min; and a final extension of 72°C for 10 min. Equal volume of PCR products were analyzed by means of electrophoresis in 1% agarose gel, and quantified using Quantity One 4.6 software.

Immunoblot assay

The levels of six mitochondrial encoded proteins both from the heavy and light chains were analyzed by means of western blot analysis. Cells transfected with pcDNA-mTERF2 or pcDNA3.1(+) empty vector were harvested by centrifugation at 36 h after the transfection, resuspended in 100 μl lysis buffer (the same with the buffer used for DNA extraction above), lyzed on ice for 10 min, and centrifuged at 14,000 *g* for 10 min at 4°C. The supernatant was mixed with 2 × SDS sample buffer, boiled for 10 min, and separated with 10% SDS-gel. The proteins in the gel were transferred to the polyvinylidene difluoride membrane (Millipore, Billerica, USA) with semi-dry transfer electrophoresis (Bio-Rad) for 50 min at 20 V. The membrane was blocked with 5% fat-free milk for 4 h at room temperature, and then incubated with the primary antibodies against mTERF2, or β-actin at a dilution of 1:1000 overnight at 4°C with gentle shake. After washing, the membrane was incubated with suitable secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, USA) at a dilution of 1:10,000 for 1 h. Finally, the signal was detected with enhanced chemiluminescence plus reagent (Beyotime, Haimen, China) and exposure to a Kodak BioMax X-ray film.

Results

mTERF2 binds to mtDNA

In preparation for ChIP assay, DNA and proteins in HeLa cells were first cross-linked with formaldehyde and then the DNA was sheared with DNase I digestion. After 1 h of DNase I digestion, the DNA fragments on agarose gel concentrated under the size of 1200 bp [**Fig. 1(A)**], which is highly desirable for this ChIP analysis.

Binding of mTERF2 to mtDNA was assayed by genome-wide ChIP assays with 17 primer pairs covering the entire mitochondrial genome [**Fig. 1(B)**]. The PCR reactions for sample input before the immunoprecipitation (IP) step with different primers resulted in different amplification efficiency but they all yielded detectable PCR products [**Fig. 1(C)**, input]. The PCR for sample after IP with anti-mTERF2 antibody with each primer pair yielded similar amount of PCR products as for the sample input [**Fig. 1(C)**, IP]. In addition, trace amount of PCR products was also detected in the sample after IP with negative antibody control [**Fig. 1(C)**, NC] due to non-specific binding of antibody to cellular proteins. No DNA product was detected in the PCR negative control sample without any templates, indicating the reliability of the PCR system [**Fig. 1(C)**, BC]. Among 17 PCR primer pairs, the ratio of PCR products from the experimental group to the sample input group showed no significant difference [**Fig. 1(D)**].

mTERF2 overexpression decreases the amount of mtDNA

The genomic DNA was extracted from HeLa cells transfected with pcDNA-mTERF2 or empty vector pcDNA3.1(+). The amount of mtDNA and nuclear genomic DNA were estimated by PCR detection of mtDNA D-Loop region and 18S rDNA, respectively (**Fig. 2**). PCR results showed that the amount of nuclear genomic DNA was not significantly different between mTERF2 overexpressed and the control cells, but the amount of mtDNA in mTERF2 overexpressed cells was significantly lower than

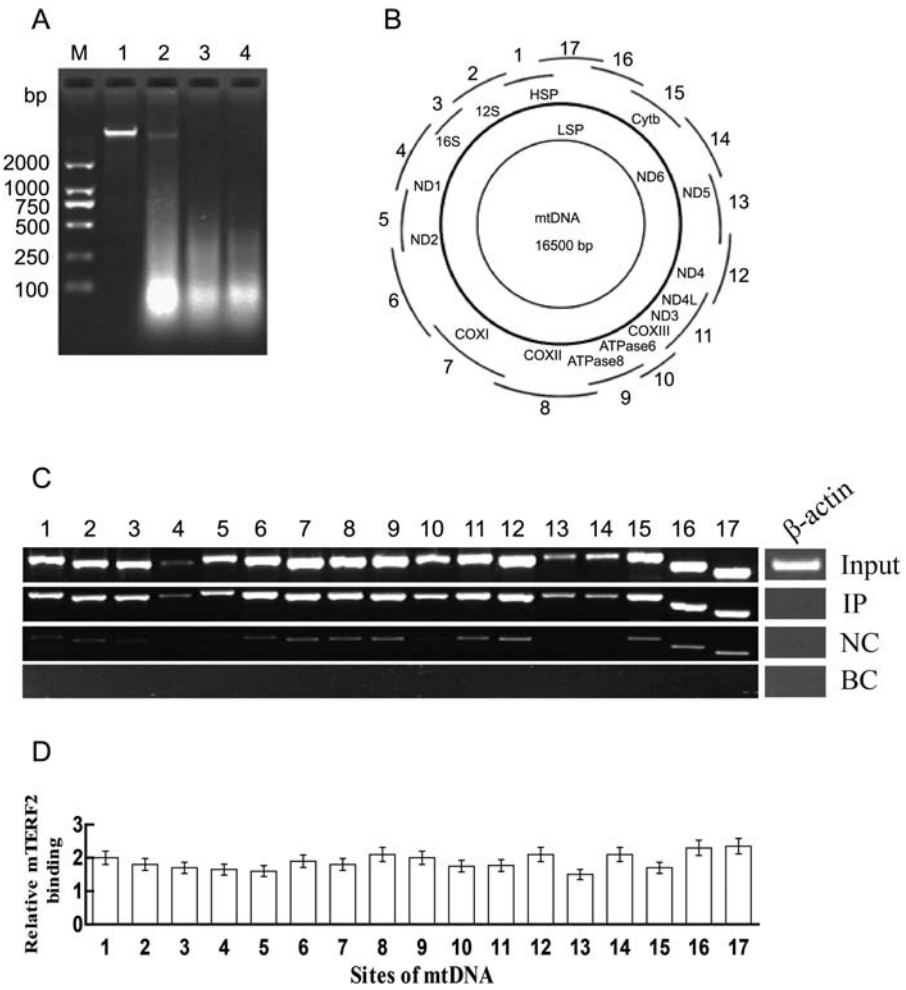

Figure 1 mTERF2 binds to the mtDNA ChIP was performed using HeLa cells and the mtDNA binding was detected by PCR. (A) DNA−protein complex was cross-linked with formaldehyde and digested by DNase I for 0, 0.5, 1 and 2 h (lanes 1–4) and the samples were analyzed by means of agarose gel electrophoresis. M: DNA ladder. (B) Primers used to detect the binding sites of mTERF2. (C) PCR results were shown using the 17 pairs of primers, and β-actin was used as a control. Input, input control; IP, immunoprecipitated; NC, no-antibody negative control; BC, no-template blank control. (D) Relative binding activity of mTERF2 on different positions of mtDNA. Data were presented as the mean ± SD of values obtained from five independent experiments.

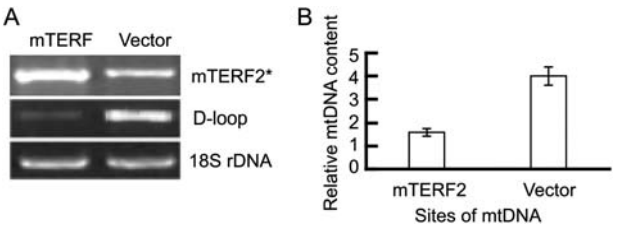

Figure 2 mTERF2 overexpression inhibits the level of mtDNA copies Total DNA was extracted from HeLa cells transfected with pcDNA-MTERF2 and pcDNA3.1(+) vector. (A) The nuclear DNA and mtDNA content were detected indirectly with amplified 18S rDNA and mtDNA D-Loop region. mTERF* was the semi-quantity RT−PCR result, which indicated the overexpression of the mTERF2 (normalized with β-actin; data not shown). (B) Relative level of mtDNA copies in mTERF2 overexpressed cells. Data were presented as the mean ± SD of values obtained from five independent experiments.

that in the control cells [**Fig. 2(A)**]. The ratio of mtDNA to the nuclear genomic DNA was also dramatically decreased in the mTERF2 overexpressed cells [**Fig. 2(B)**].

mTERF2 overexpression decreases the level of mitochondrial mRNA

Five heavy-chain genes (*12S rRNA*, *16S rRNA*, *ND1*, *COXII*, and *Cytb*) and two light-chain genes (*ND6* and the transfer RNA of amino acid Q) were selected to represent mitochondrial genes, and their mRNA levels were qualified by semi-quantitative RT−PCR (**Fig. 3**). From the same cells transfected with pcDNA-mTERF2 or the empty vector, overexpression of mTERF2 significantly reduced the mRNA level of all these mitochondrial genes, while the levels of β-actin remained unchanged [**Fig. 3(A)**]. The fold decrease was in the same magnitude with the decrease in the mtDNA content [**Fig. 3(B)**].

mTERF2 overexpression decreases the translation level of mitochondrial proteins

From the same cells transfected with pcDNA-mTERF2 or the empty vector, five proteins in the heavy chain (ND1, ND4,

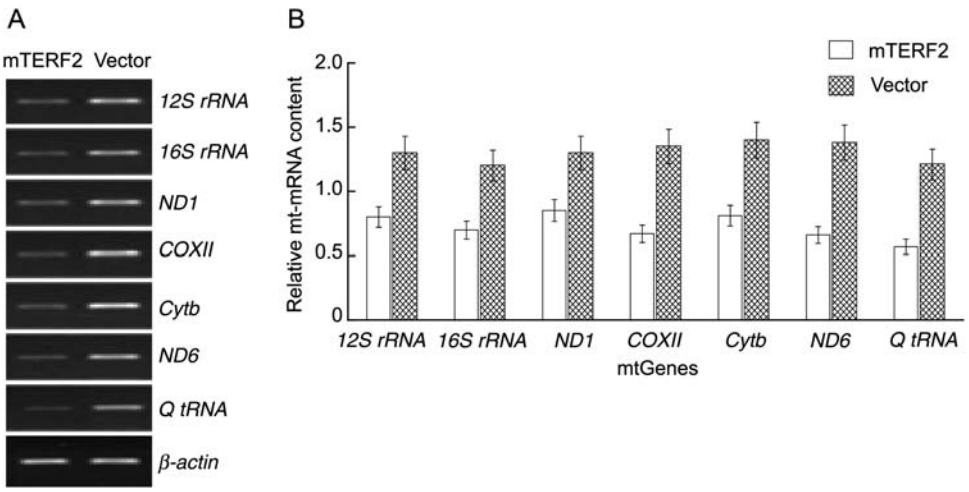

Figure 3 mMTERF2 overexpression decreases mRNA level of mitochondrial genes HeLa cells transfected with pcDNA-MTERF2 and pcDNA3.1(+) vector. (A) Gel images of RT−PCR. β-actin was employed as a loading control. (B) Relative levels of mitochondrial mRNAs in mTERF2 overexpressed cells. Data were presented as the mean ± SD of values obtained from three experiments.

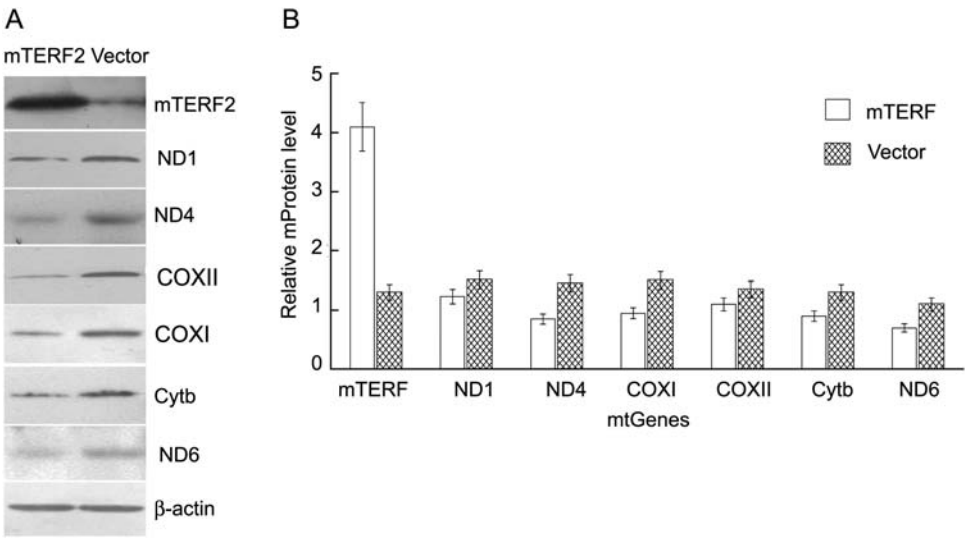

Figure 4 Overexpression of mTERF2 inhibits mitochondrial protein synthesis The expression of mTERF2 and selected mitochondrial proteins of the transfected cells were examined by western blot analysis. (A) Results were presented as the images from western blot analysis, and β-actin was used as the loading control. (B) Relative intensity of mTERF2 and mitochondrial protein levels. Data were presented as the mean ± SD of values obtained from three experiments.

COXII, Cytb, and COXI) and one in the light chain (ND6) of mitochondria were selected to represent mtDNA-encoded proteins, and their levels were measured by western blot analysis (**Fig. 4**). Although mTERF2 was overexpressed, the levels of all the six mitochondrial proteins were decreased compared with those in the control cells [**Fig. 4(A)**]. The fold changes in the protein levels were similar to those of mitochondrial mRNA transcripts [**Fig. 4(B)**].

All the above experiments were also carried out in U251 and MCF-7 cell lines, and similar results were obtained (data not shown).

Discussion

mTERF2 is known as an important nuclear protein that interacts with mtDNA, and different modes of mTERF2 interaction with mtDNA have been reported. Some reports showed that mTERF2 bound specifically to the HSP region and was a positive regulator of mitochondrial transcription [12,22]; others showed that mTERF2 was a mitochondrial protein and displayed a non-specific mtDNA-binding activity that was abundantly detectable *in vivo*, with one monomer present per 265 bp of mtDNA [10]. In addition,

the binding of mTERF2 to mitochondrial D-Loop region and encoding sequence were assayed by electrophoretic mobility shift assay and the results showed that mTERF2 binding to the mtDNA was non-specific [11]. In our ChIP analysis, all the mtDNA fragments were co-precipitated equally with mTERF2 antibody, suggesting that the mTERF2 bound to the entire mtDNA in the similar efficiency *in vivo*. Since the DNA fragments in the ChIP assay were around 1 kb, there should be at least one mTERF2 binding site in each 1000 bp of mtDNA. Our results supported the possibility that mTERF2 randomly bound to the entire mtDNA with no specificity.

Two models have been proposed for the replication of mtDNA in mammals. One is the strand asynchronous model in which replication occurs unidirectionally with the synthesis of the daughter strand from heavy strand starting at O_H. When replication of heavy strand reaches 2/3 of the genome, O_L is activated and the replication of the light chain starts in the opposite direction [23]. The other is the strand synchronous model suggesting that replication occurs symmetrically in the leading-lagging strand and starting from different points [24]. In either the asynchronous or synchronous model, replication is initiated at special sites. From this study, since mTERF2 showed no special binding pattern on mtDNA, and it negatively regulated the level of mtDNA, it might regulate the replication process rather than its initiation, as the histone bound to the genomic DNA [10].

Although the binding pattern made mTERF2 unlikely serve to directly regulate the transcription or translation of mitochondrial genes, its wide range of binding to mtDNA suggested an important function of this protein. In the present study, we first found that overexpression of mTERF2 decreased the level of mitochondrial mRNA and proteins in various cell lines. Since the decrease folds of mRNA and protein levels are similar to that of mtDNA level, decreases at mRNA and protein levels of mitochondrial genes were most likely resulted from the overall decrease of mtDNA.

Previous reports showed that mTERF2 inhibited cell growth [13,25], but the mechanism was not clear. Mitochondrion is the cell's power plant responsible for energy generation. Its activities are largely dependent on the regulation of mitochondrial gene expression [26,27]. Based on our results, mTERF2 might exert its growth inhibitory function by reducing the mtDNA copies, thus decreasing the overall mitochondrial protein levels. Because human mtDNA encodes 13 mitochondrial proteins that make up all subunits of OXPHOS complex, the decrease of overall mitochondrial protein synthesis could reduce the assembly of OXPHOS complex, and then generate insufficient ATP for the cell growth [27,28]. Therefore,

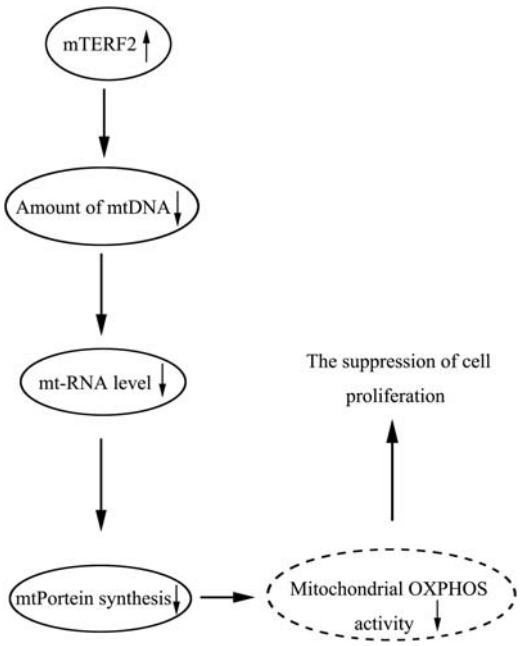

Figure 5 Potential mechanism of MTERF2 on regulating cell proliferation

it can be one of the potential mechanisms on how mTERF2 inhibits cell proliferation (**Fig. 5**).

Wenz *et al*. [12] constructed the mTERF2 knockout mice, and found that the mitochondrial protein synthesis level in fibroblasts was decreased when mTERF2 was lost. And mitochondrial transcripts of the challenged mTERF2 knockout mice muscle showed marked decreases for both heavy- and light-strand transcripts. These aberrations were associated with decreased steady-state levels of OXPHOS proteins causing a decrease in respiratory function [12]. It suggested that mTERF2 was an up-regulator of mitochondria genes expression. Hyvarinen *et al*. [11] reported that overexpression of epitope-tagged MTERFD1 (mTERF3) or MTERFD3 (mTERF2) resulted in modest mtDNA copy-number depletion in 293T cells. And mtDNA copy number decrease will reduce the mRNA level, which showed that mTERF2 was a negative regulator of mitochondria gene expression similar to our results. These researches indicated that mTERF2 might play different roles in human and mice or in different physiological states. More studies are needed to consolidate these results and to clarify the function of mTERF2.

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