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Human mitochondrial transcription factor A functions in both nuclei and mitochondria and regulates cancer cell growth

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ABSTRACT

Mitochondrial transcription factor A (mtTFA) is one of the high mobility group protein family and is required for both transcription from and maintenance of mitochondrial genomes. However, the roles of mtTFA have not been extensively studied in cancer cells. Here, we firstly reported the nuclear localization of mtTFA. The proportion of nuclear-localized mtTFA varied among different cancer cells. Some mtTFA binds tightly to the nuclear chromatin. DNA microarray and chromatin immunoprecipitation assays showed that mtTFA can regulate the expression of nuclear genes. Overexpression of mtTFA enhanced the growth of cancer cell lines, whereas downregulation of mtTFA inhibited their growth by regulating mtTFA target genes, such as baculoviral IAP repeat-containing 5 (*BIRC5*; also known as survivin). Knockdown of mtTFA expression induced p21-dependent G1 cell cycle arrest. These results imply that mtTFA functions in both nuclei and mitochondria to promote cell growth.

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1. Introduction

Mitochondrial transcription factor A (TFAM; also known as mtTFA) is a member of the high mobility group (HMG) box protein family and activates transcription from the D-loop region of the mitochondrial genome [1–3]. Mitochondria play a critical role in cancer cell metabolism, which is also essential for cell proliferation [4]. It is well known that mitochondrial uncoupling mediates the metabolic shift to aerobic glycolysis in cancer cells [4,5]. Thus, mitochondria control cell survival and growth. In addition, the number of mitochondria correlates with the growth rate of cancer cells [6,7].

mtTFA-null mice show an embryonic lethal phenotype and apoptosis in heart cells [8]. Nuclear HMG box proteins such as HMGB1/B2 are often overexpressed in cancer cells, bind preferentially to p53 and are involved in apoptosis [9,10]. mtTFA protein multimerizes and binds to mitochondrial DNA, indicating that the mtTFA levels might be increased in cancer cells and be related to malignant progression and proliferative activity.

Prostate cancer is the most prevalent form of cancer among men. Microscopic prostate cancers can be detected in 12.4% elderly men in USA, indicating an association of the cancer with aging and oxidative stress [11]. We previously found that mtTFA is overexpressed in cisplatin-resistant human cancer cells [12]. mtTFA may function to protect mitochondrial DNA from oxidative stress. It has been shown that high expression of mtTFA correlated with poor prognosis in endometrial carcinoma [13] and colon cancer [14]. These data are in accordance one of the hallmarks of cancer: the evasion of apoptosis.

Interestingly, we found that mtTFA was detected within the nuclear chromatin of cancer cells and may regulate nuclear gene expression in addition to its role as a transcription factor in mitochondria.

Abbreviations: BIRC5, baculoviral IAP repeat containing 5; ChIP, chromatin immunoprecipitation; HMG, high mobility group; mtDNA, mitochondrial DNA; MTP, mitochondrial targeting peptide; mtTFA/TFAM, mitochondrial transcription factor A; NLS, nuclear localization signal; PRDX3, peroxiredoxin 3; siRNA, small interfering RNA; YBX1, Y box binding protein 1.

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2. Materials and methods

2.1. Cell culture and antibodies

Human prostate cancer cell line PC3 was obtained from the American Type Culture Collection. The human clear cell renal cancer SKR1 and Caki-1 cells were kindly provided by Dr. Seiji Naito (Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan). Antibodies to p21 (sc-187), baculoviral IAP repeat-containing 5 (BIRC5; also known as survivin) (sc-10811) and β-tubulin (sc-5274) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Flag (M2) and anti-β-actin (AC-15) antibodies were purchased from Sigma (St. Louis, MO). Polyclonal antibodies against mtTFA [15], BRG1-associated factor 57 (SMARCE1, also known as BAF57) [16] and HMGB1 [17] have been described previously. Anti-peroxiredoxin 3 (PRDX3) antibody was a kind gift from Hiroki Nanri (Seinan Jogakuin University, Fukuoka, Japan).

2.2. Plasmid preparation

Human mtTFA cDNA without a stop codon was amplified by reverse-transcription PCR from total RNA extracted from HeLa cells with the primer pair: 5′-CGATGGCGTTTCTCCGAAGC-3′ and 5′-ACACTCCTCAGCACCATATTTTCG-3′. The underline indicates the start codon. To obtain the mtTFA-3×Flag expression plasmid (pcDNA3-mtTFA-3×Flag), the mtTFA cDNA fragment containing 3×Flag sequences at the C-terminus was ligated into a pcDNA3.1/hygromycin mammalian expression plasmid (Invitrogen, Carlsbad, CA). To construct BIRC5-luciferase reporter plasmid, putative BIRC5 promoter region (−992 to +110) was amplified by PCR using the human genomic DNA and the following primer pairs: GGGAGGGGTGGGGAGAGGTTGC and AAGCTTCGCGGGACCCGTT GGCAGAGGTG. Underlining indicates the HindIII restriction enzyme cleavage site. PCR product was cloned and ligated into the EcoRV–HindIII site of the pGL4.16 luc2CP/Hygro vector (Promega, Madison, WI).

2.3. Cloning of stable transfectants

The cloning of stable transfectants was described previously [18]. Briefly, PC3 cells were transfected with pcDNA3-mtTFA-3×Flag with Superfect Transfection Reagent (Qiagen, Valencia, CA) and cultured with medium containing 500 μg/ml hygromycin for 20 days. The resulting colonies were isolated and the expression level of mtTFA was investigated by Western blotting with the anti-mtTFA antibody. Two stable transfectants named as mtTFA-3F CL1 and CL9 were established.

2.4. Cell fractionation

Cell pellets were resuspended in hypotonic buffer A, containing 10 mM Hepes–KOH, pH 7.9, 10 mM KCl, 0.1 mM EDTA–NaOH, pH 8.0, 0.1 mM EGTA, 1 mM dithiothreitol (DTT) and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and incubated for 15 min on ice. After addition of Nonidet-P40 to 0.3% final concentration, cells were gently resuspended and centrifuged at 4200g for 5 min. The supernatant was stored for the cytoplasmic fraction (CF). The nuclear pellet was resuspended in high salt buffer C (1/4 volume of buffer A), containing 20 mM Hepes–KOH, pH 7.9, 0.4 M NaCl, 1 mM EDTA–NaOH, 1 mM EGTA, 1 mM DTT and 0.5 mM PMSF. The half volume of buffer C containing nucleus was sonicated for 20 s for nuclear fraction (NF). The remained half volume of nuclei in buffer C was incubated for 30 min on ice and centrifuged at 21,000g for 10 min. The supernatant was stored for use as the nuclear extract (NE), i.e., proteins that loosely bind to DNA. The pellet was sonicated in buffer C (the same volume of NE) for 20 s and stored for use as the chromatin fraction (ChF), i.e., proteins that tightly bind to DNA. For preparation of whole cell lysate, the cell pellet was sonicated in buffer A for 20 s.

2.5. Indirect immunofluorescence

PC3 cells were divided into cytoplasm and nuclei as described above. Nuclei were suspended with 15% formalin neutral buffer solution (Wako, Osaka, Japan) for 10 min and mounted on glass coverslips coated with poly-L-lysine (3438-100-01, R&D Systems) and were permeabilized with PBS containing 0.2% Triton X-100 for 10 min at room temperature, and then incubated with normal serum or serum containing anti-mtTFA antibody (1:200 dilution) in PBS including 0.3% BSA for 60 min at room temperature. After washing three times with PBS, the samples were incubated with fluorescein-conjugated goat anti-rabbit IgG (Alexa Fluor 488; Molecular Probes, OR) and 4,6-diamidino-2-phenylindole (DAPI; Sigma) in PBS including 0.3% BSA for 45 min at room temperature. The samples were washed three times with PBS and mounted directly on slides with Gel/Mount (M01, Biomeda). Digital photographs were taken with a Nikon ECLIPSE E600 fluorescence microscope and DS-5M, -L1 (Nikon, Tokyo, Japan). Exposure time was fixed in each picture.

2.6. Western blotting

Western blotting was described previously [18]. For calculating the ratio between fractionations, indicated amounts of volume were subjected by Western blotting. Detection was performed using enhanced chemiluminescence (Amersham, Piscataway, NJ). The protein expression levels were quantitated using a Multi Gauge Version 3.0 (Fujifilm, Tokyo, Japan).

2.7. Knockdown analysis using small interfering RNAs (siRNAs)

Knockdown using siRNAs was performed as described previously [19]. The following 25-bp, double-stranded RNA oligonucleotides were commercially generated (Invitrogen) 5′-UGCACAGCUCUGCUCCAGACCUUCC-3′ and 5′-GGAAGGUCUGGA GCAGAGCUGUGCA-3′ for *mtTFA*-siRNA (siTFAM) #1, 5′-AUACCU GCCACUCCGCCCUAUAAGC-3′ and 5′-GCUUAUAGGGCGGAGU- GG-CAGGUAU-3′ for siTFAM #2.

2.8. Cell proliferation assay

Cell proliferation assays were performed as described previously [19].

2.9. Flow cytometry

Flow cytometry assays were performed as described previously [20].

2.10. Chromatin immunoprecipitation (ChIP) assay

The ChIP assay with transient transfection and anti-Flag antibody was described previously [21]. The purified DNA was used for PCR analysis with the following primer pairs: 5′-GGGAG-GGGTGGGGAGAGGTTGC-3′ and 5′-CGCTGTCCCGGCGAGTACATCG-3′ for the *BIRC5* promoter, 5′-GCCCGGCACTACGGGCTGCG-3′ and 5′-GTGTGCGCAGGCCGCGGACG-3′ for the Y-box binding protein 1 (*YBX1*) gene. The PCR products were separated by electrophoresis on 2% agarose gels and stained with ethidium bromide.

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