p53/PGC-1α-mediated mitochondrial dysfunction promotes PC3 prostate cancer cell apoptosis

JIULING LI[1], YANY LI[1], LANLAN CHEN[2], BINGBING YU[1], YANAN XUE[1], RUI GUO[1], JING SU[1], YANAN LIU[1] and LIANKUN SUN[1]

[1]Department of Pathophysiology, Prostate Diseases Prevention and Treatment Research Center, College of Basic Medical Sciences; [2]School of Clinical Medicine, Jilin University, Changchun, Jilin 130021, P.R. China

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Abstract. Data for p53 mutation in prostate cancer in The Cancer Genome Atlas database revealed that >85% of p53 mutations occurred in the p53 DNA binding domain. These mutations not only severely damage the function of the p53 protein, but also reduce the disease-free survival of patients. Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is involved in the regulation of mitochondrial function and is highly expressed in prostate cancer PC3 and DU145 cells with p53 deletion or mutation. However, whether p53 negatively regulates PGC-1α in prostate cancer cells remains to be elucidated. In the present study, p53 overexpression was induced in prostate cancer PC3 cells. Subsequently, the expression levels of PGC-1α and alterations to mitochondrial function were assessed. Moreover, PGC-1α was activated in prostate cancer PC3 cells using ZLN005 to investigate alterations to mitochondrial function and cell apoptosis. The present study revealed that p53 decreased the expression and nuclear localization of the PGC-1α protein and induced mitochondrial dysfunction. Activation of PGC-1α partially reversed p53-mediated mitochondrial dysfunction. Inhibition of the p53/PGC-1α pathway on mitochondrial biogenesis and fission-/fusion-associated gene and protein expression were associated with mitochondrial dysfunction. p53/PGC-1α-mediated mitochondrial dysfunction promoted apoptosis of PC3 prostate cancer cells. The results indicated that PGC-1α is an essential target of p53-induced apoptosis in prostate cancer cells and indicated that targeting PGC-1α may provide a new therapeutic strategy for prostate cancer.

Correspondence to: Professor Liankun Sun or Professor Yanan Liu, Department of Pathophysiology, Prostate Diseases Prevention and Treatment Research Center, College of Basic Medical Sciences, Jilin University, 126 Xinmin Street, Chaoyang, Changchun, Jilin 130021, P.R. China
E-mail: sunlk@jlu.edu.cn
E-mail: ynliu@jlu.edu.cn

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Introduction

Prostate cancer is the most common malignant tumor in men, with a mortality rate second to lung cancer (1). Nearly 1.3 million new prostate cancer cases and 359,000 associated deaths were reported worldwide in 2018 (2). Although surgery or radiotherapy can effectively improve the prognosis and prolong the survival of prostate cancer patients, the recurrence and metastasis rates remain high. Therefore, identification of novel potential anticancer targets for the development of therapeutic treatments is critical.

p53 is a classic tumor suppressor. Overexpression of p53 not only inhibits the growth of prostate cancer cells but also enhances the chemosensitivity of prostate cancer cells (3,4). Several studies have demonstrated that peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is expressed at high levels in p53-mutant or p53-deleted tumor cells and have revealed that PGC-1α plays a role in cancer progression. For example, Shiota *et al* (5) reported that PGC-1α was highly expressed in prostate cancer PC3 and DU145 cells with p53 deletion or mutation and promoted tumor cell growth. Ogasawara *et al* (6) revealed that PGC-1α was highly expressed in p53-deficient chronic lymphocytic leukemia cells and maintained normal mitochondrial function. The R72 variant of mutant p53 promoted tumor metabolism and metastasis by enhancing the function of PGC-1α (7). These findings indicate that the deletion or mutation of p53 may contribute to the enhancement of PGC-1α expression and function in tumor cells. However, the effect of wild-type p53 on PGC-1α in tumor cells is unclear.

PGC-1α is a member of the peroxisome proliferator-activated receptor γ coactivator 1 family that coordinates the activity of transcription factors to modulate energy metabolism and other cellular processes (8). As a primary regulator of mitochondrial biogenesis, PGC-1α functions in the activation of the nuclear respiratory factor 1 (NRF1) transcription factor, leading to the transcription of both nuclear-encoded mitochondrial genes (such as *SDHA*) and the transcription factor A, mitochondrial (*TFAM*) (9,10). PGC-1α is also involved in the regulation of mitochondrial fission/fusion; PGC-1α promotes mitochondrial fusion through transcriptional activation of mitofusin (*MFN*) 1 and 2 and affects mitochondrial fission via direct regulation of dynamin-related

protein 1 (DRP1) expression by binding to its promoter (11-14). Peng *et al* (15) revealed that rotenone reduces TFAM, MFN2 and DRP1 expression by inhibiting PGC-1α in pheochromocytoma PC12 cells, resulting in decreased mitochondrial copy number, imbalance of fission/fusion and cell death. Thus, PGC-1α-mediated regulation of mitochondrial biogenesis and fission/fusion not only affects mitochondrial function but also determines the survival and death of tumor cells. These findings indicate that PGC-1α may be a potential target for cancer therapy.

The present study examined the effect of p53 on PGC-1α and revealed that p53 decreased the expression of mitochondrial biogenesis and fission/fusion-associated genes by inhibiting PGC-1α, leading to mitochondrial dysfunction and ultimately apoptosis. These findings revealed that PGC-1α is a crucial target of p53-induced apoptosis in PC3 prostate cancer cells and indicated that targeting PGC-1α may provide a new therapeutic strategy for prostate cancer.

Materials and methods

Cell culture and reagents. Human prostate cancer cell lines PC3 and DU145 were purchased from the American Type Culture Collection and cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc.). ZLN005, a transcriptional activator of PGC-1α, was purchased from MedChemExpress LLC. Hoechst 33342 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Merck KGaA). Anti-β-actin (cat. no. sc-47778), anti-p53 (cat. no. sc-6243), anti-Mfn1 (cat. no. sc-166644), anti-Mfn2 (cat. no. sc-100560), anti-DRP1 (cat. no. sc-271583), anti-Bak (cat. no. sc-832) and anti-Bcl-2 (cat. no. sc-509) antibodies were purchased from Santa Cruz Biotechnology, Inc. Anti-NRF1 (cat. no. A5547) and anti-TFAM (cat. no. A1926) antibodies were purchased from ABclonal Biotech Co., Ltd. Anti-PGC-1α (cat. no. 66369-Ig) and anti-SDHA (cat. no. 14865-1-AP) antibodies were purchased from ProteinTech Group, Inc. Anti-cleaved-caspase-3 (product no. 9664) was purchased from Cell Signaling Technology, Inc.

Transfection and drug treatment. The pcDNA3.1 vector (negative control) and the full-length p53 expression vector were purchased from Shanghai GeneChem Co., Ltd. Transfections were performed using TurboFect Transfection Reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Briefly, PC3 cells were seeded ($5x10^5$ cells/well) into 6-well plates and cultured overnight at 37˚C. Subsequently, cells were transfected with p53 expression vector (4 μg) or pcDNA3.1 (4 μg). Following transfection for 4-6 h at 37˚C, the PGC-1α activator ZLN005 was added and the cells were cultured for 24 h at 37˚C prior to subsequent experiments. shRNA sequences targeting human PGC-1α and a non-target sequence were constructed by GeneChem Co., Ltd. (Shanghai, China.), as previously described (16). The sequences of PGC-1α shRNA and the non-target shRNA were 5'-GTTATACCTGTGATGCTTT-3' and 5'-TTCTCCGAA CGTGTCACGT-3', respectively. Cells were transfected with shRNAs (4 μg) using the GV248 vector (GeneChem, Inc.), according to the aforementioned protocol.

Immunofluorescence staining and confocal laser microscopy. Coverslips were placed in 24-well plates and $8x10^4$ cells were added into each well. The transfection efficiency of the p53 plasmid and the co-localization of p53 with PGC-1α were examined using indirect immunofluorescence, as previously described (17). Stained cells were observed in five random fields of view using an FV1000 confocal laser microscope (Olympus Corporation; magnification, x200).

Reverse transcription-quantitative (RT-q) PCR. Cells were cultured in 6-well plates at a density of $5x10^5$ cells/well. Total RNA was extracted from cells using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Subsequently, total RNA was reverse transcribed into cDNA using 0.5 μg total RNA and the SuperScript Pre-amplifcation system (Promega Corporation), according to the manufacturer's protocol. qPCR was performed using the CFX96 Touch™ Real-Time PCR detection system (Bio-Rad Laboratories, Inc.), as previously described (15), and the TransStart Top Green qPCR SuperMix (cat. no. AQ131; Beijing Transgen Biotech Co., Ltd.), with a reaction system consisting of 1 μl cDNA, 0.2 μM forward primer, 0.2 μM reverse primer, 10 μl qPCR SuperMix and 8.6 μl nuclease-free water. The following thermocycling conditions were used for qPCR: Initial denaturation at 94˚C for 30 sec, 40 cycles of 94˚C for 5 sec and a final extension at 60˚C for 30 sec. The following primer pairs were used for qPCR: *NRF1* forward, 5'-GCTACTTACACCGAGCAT AGTA-3' and reverse, 5'-CTCAAATACATGAGGCCG TTTC-3'; *TFAM* forward, 5'-TTCCAAGAAGCTAAGGGT GATT-3' and reverse, 5'-AGAAGATCCTTTCGTCCAACT T-3'; *SDHA* forward, 5'-GTCCCTCCAATTAAACCAAAC G-3' and reverse, 5'-GTTCCGATGTTCTTATGCTTCC-3'; *MFN1* forward, 5'-GTGGCAAACAAAGTTTCATGT G-3' and reverse, 5'-CACTAAGGCGTTTACTTCATCG-3'; *MFN2* forward, 5'-GTGCTTCTCCCTCAACTATGAC-3' and reverse, 5'-ATCCGAGAGAGAAATGGAACTC-3'; *DRP1* forward, 5'-GAGATGGTGTTCAAGAACCAAC-3' and reverse, 5'-CAATAACCTCACAATCTCGCTG-3'; and β-actin forward, 5'-CCTGGCACCCAGCACAAT-3' and reverse, 5'-GGGCCGGACTCGTCATAC-3'. mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (18) and normalized to the internal reference gene β-actin.

Western blot analysis. Cells were lysed using RIPA lysis buffer (Takara Bio, Inc.), sonicated at 20 kHz for 30 sec on ice and incubated on ice for 45 min. Cell lysates were centrifuged at 3,000 x g for 15 min at 4˚C. Total protein was quantified using the Coomassie G250 assay (Beyotime Institute of Biotechnology). Protein samples (20-30 μg) were separated by 12% w/v SDS-PAGE and transferred onto PVDF membranes. The PVDF membranes were blocked with 5% skim milk powder for 2 h at room temperature. Subsequently, the membranes were incubated overnight at 4˚C with primary antibodies (primary antibodies purchased from Santa Cruz Biotechnology, Inc. were diluted at 1:200; primary antibodies purchased from ProteinTech Group, Inc. or Cell Signaling Technology, Inc. were diluted at 1:1,000.). Anti-β-actin was use as loading control. Following primary incubation, the membranes were incubated with horseradish-peroxidase-conjugated secondary

antibodies (1:2,000; cat.no. SA00001-1 and SA00001-2; ProteinTech Group, Inc.) for 2 h at room temperature. Protein bands were visualized using Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific, Inc.) and imaged using a Syngene BioImaging system (Synoptics). Protein expression was quantified using ImageJ software (version 1.48; National Institutes of Health) with β-actin as the loading control.

Oxygen consumption rate (OCR). OCR was measured using the MitoXpress® Xtra-Oxygen Consumption assay (Luxcel Biosciences Ltd.) as previously reported (19).

Mitochondrial mass. Coverslips were placed in 24-well plates and $8x10^4$ cells were added into each well. Mitochondria were stained in live cells using MitoTracker™ Red CMXRos (Invitrogen; Thermo Fisher Scientific, Inc.), as previously described (16).

Cell viability assays. Cells were cultured ($1.2x10^4$ cells/well) in 96-well plates overnight at 37˚C and subsequently cultured for 24 h at 37˚C after the addition of various concentrations of ZLN005 (5,10,15 or 20 μM). MTT assays were performed as previously described (19). Briefly, 10 μl MTT (10 mg/ml) reagent was added to each well and incubated for 4-6 h. The formazan crystals were dissolved with 150 μl DMSO and the absorbance was measured at a wavelength of 490 nm.

Annexin V and cell death assay. Cells were cultured in 6-well plates ($5x10^5$ cells/well) and transfection was performed within 24 h. At 4-6 h post-transfection, ZLN005 (15 μM) was added to the culture. After incubation for 24 h at 37˚C, the Annexin V Apoptosis Detection Kit II (BD Biosciences) was used to measure cellular apoptosis, according to the manufacturer's protocol. Apoptotic cells were detected by flow cytometry using a BD Accuri C6 cytometer (BD Biosciences), and data analysis was conducted using BD Accuri C6 software (version 1.0.264.21; BD Biosciences).

Dataset analysis. All dataset analysis results were obtained from cBioPortal (http://www.cbioportal.org/), which hosts multiple cancer genomics studies, including all of the data from The Cancer Genome Atlas (TCGA). Patient survival was determined by Kaplan-Meier and log-rank analyses and correlation analysis was determined by Spearman and Pearson methods.

Statistical analysis. Each experiment was repeated three times. Results are expressed as the mean ± standard deviation. One-way analysis of variance was performed to compare results among the groups, followed by Bonferroni's multiple comparison test to determine statistical significance. All statistical analyses were performed using SPSS 24 statistical software (IBM Corp.).

Results

p53 mutation is associated with decreased disease-free survival in prostate cancer patients. The TCGA data demonstrated that 12% of prostate cancer patients harbor p53 mutations (Fig. 1A). Most of these mutations occur in the p53 DNA-binding domain and severely impair the function of the p53 protein (Fig. 1B). Survival analysis demonstrated no significant difference in overall survival between patients with p53 mutations and p53 non-mutation patients, but the disease-free survival rate of patients with p53 mutations was decreased compared to p53 non-mutation patients (Fig. 1C). Unlike p53, the mutation rate of PGC-1α in prostate cancer patients was only 0.8% and these mutations had a weak effect on the function of PGC-1α (Fig. S1A); therefore, the finding that PGC-1α mutation reduced the survival rate requires verification using a larger sample size (Fig. S1B).

p53 inhibits the protein expression and nuclear localization of PGC-1α. The deletion or mutation of p53 may contribute to the enhancement of PGC-1α expression and function. However, whether p53 negatively regulates PGC-1α is unclear. To explore the effect of wild-type p53 on PGC-1α, p53-deleted PC3 prostate cancer cells were transfected with a construct that overexpresses p53. Immunofluorescence staining (Fig. 2A) and western blot analysis (Fig. 2B) confirmed that p53 was successfully overexpressed in PC3 cells transfected with the p53 overexpression vector, while no p53 was detected in negative control (NC) cells. Next, the protein expression and nuclear localization of PGC-1α in response to p53 overexpression was examined. As revealed in Fig. 2C, a decrease in PGC-1α protein expression was detected in p53-overexpressing cells compared with the NC cells. Furthermore, immunofluorescence assay demonstrated that the nuclear staining of PGC-1α observed in NC cells was decreased upon p53 overexpression (Fig. 2D). These results indicated that p53 inhibited the protein expression and nuclear localization of PGC-1α. In addition, correlation analysis demonstrated that p53 and PGC-1α tended to be negatively associated, which is consistent with the results of the present study, although the P-value was not significant (Fig. S1C).

Inhibition of PGC-1α is involved in p53-induced mitochondrial dysfunction. Lack of PGC-1α has been reported to be a major cause of mitochondrial dysfunction (20). OCR and mitochondrial mass were examined to determine the effect of p53 on mitochondrial function. The effect of p53 on OCR in PC3 cells was examined and there was a decrease in OCR by 52% in PC3 cells transfected with the p53 overexpression vector compared with NC cells (Fig. 3A). To evaluate the effect of p53 on mitochondrial mass, PC3 cells were stained with MitoTracker Red, a fluorescence dye that stains mitochondria in a mass-dependent fashion. A marked decrease in mitochondrial mass in p53-overexpressing PC3 cells compared with NC cells was observed (Fig. 3B). These results confirmed that p53 induced mitochondrial dysfunction in PC3 cells.

To examine whether p53-induced mitochondrial dysfunction involves inhibition of PGC-1α, the mitochondrial function in PC3 cells after activation of PGC-1α by ZLN005 was examined. First, PC3 cells were treated with various concentrations of ZLN005. MTT assays indicated that treatment of PC3 cells with ZLN005 at concentrations of ≤15 μM resulted in increased cell survival (Fig. 3C). Next, PGC-1α expression was examined by western blotting. The results indicated that the levels of the PGC-1α protein were significantly increased as the concentration of ZLN005 increased (Fig. 3D and E). A concentration of 15 μM, which demonstrated beneficial effects on cell survival,

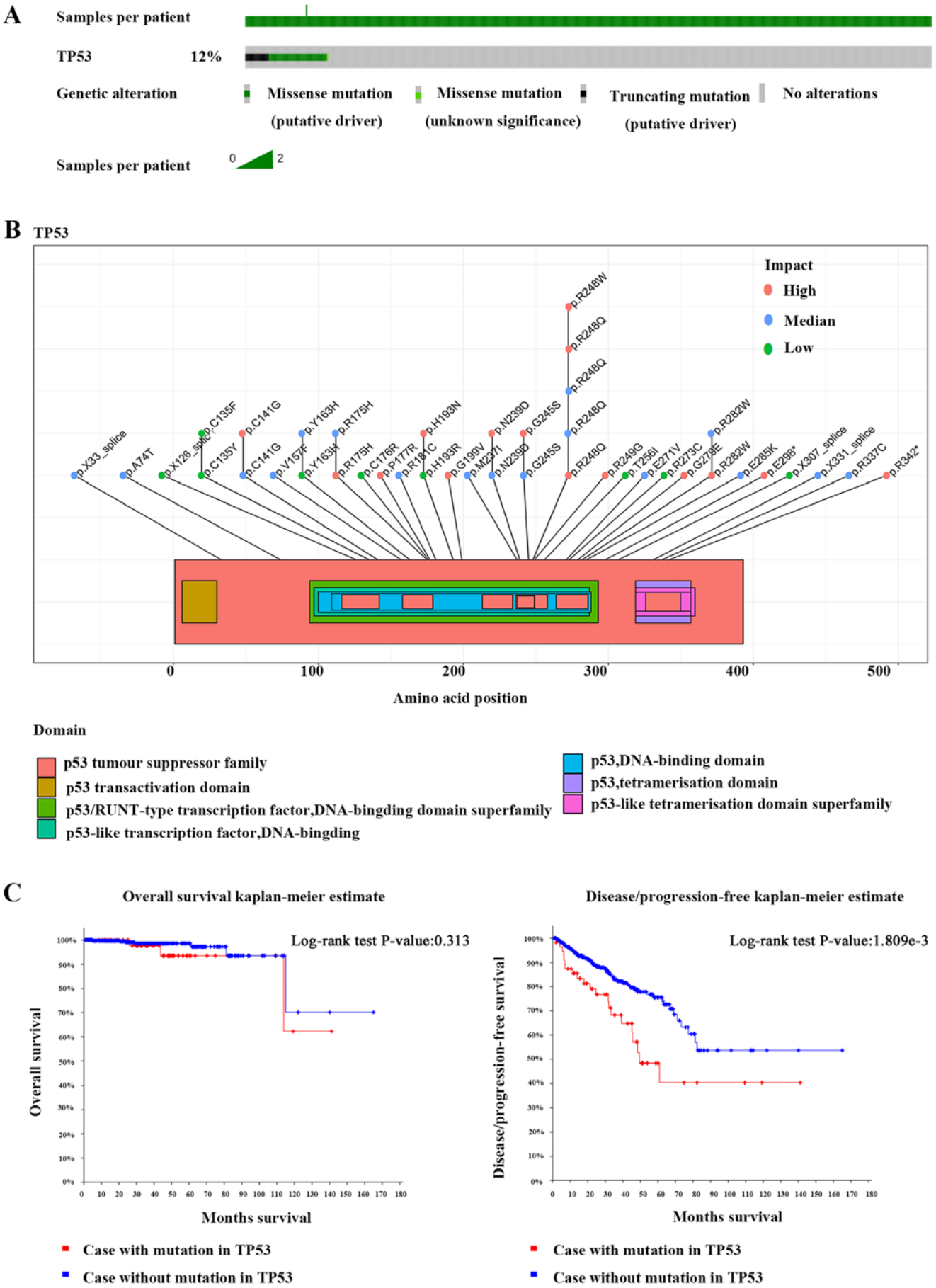

Figure 1. p53 mutation is associated with reduced disease-free survival in prostate cancer patients. All datasets can be obtained from cBioPortal (http://www.cbioportal.org/), which hosts multiple cancer genomics studies, including all of the data from The Cancer Genome Atlas. (A) Mutation frequency of p53 in patients with prostate cancer. (B) p53 mutation site and effects on p53 function. (C) Survival analysis of prostate cancer patients with and without p53 mutation.

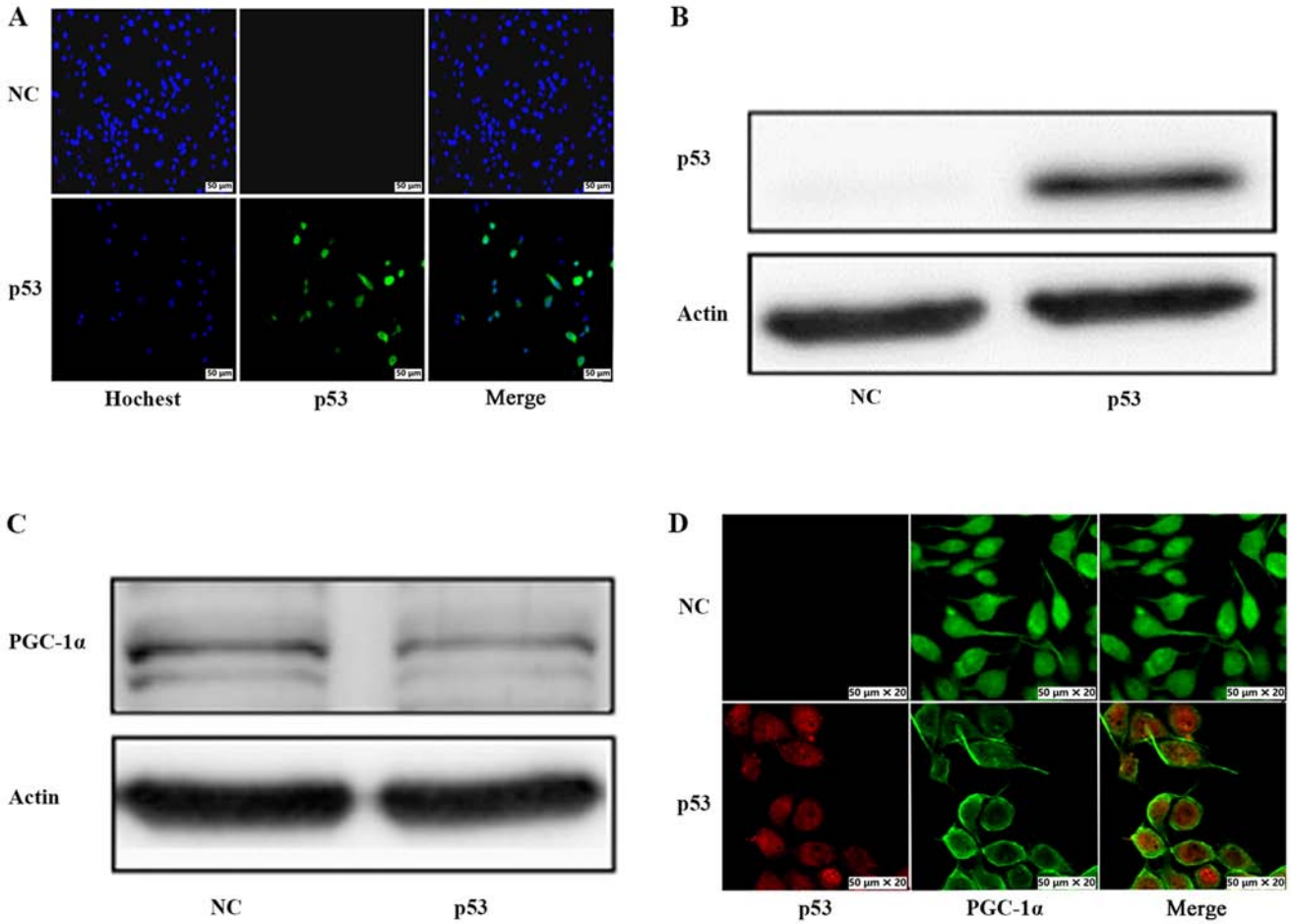

Figure 2. p53 inhibits protein expression and nuclear localization of PGC-1α. PC3 cells were transfected with a p53 overexpression construct for 24 h. (A) Immunofluorescence staining and (B) western blot analysis were used to analyze the efficiency of p53 overexpression. The expression and nuclear localization of PGC-1α were analyzed by (C) western blot analysis and (D) immunofluorescence staining. NC, negative control; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

was selected for subsequent experiments. p53-overexpressing cells were treated with ZLN005 and the effects on OCR and mitochondrial mass were examined. The OCR was increased by 30% in PC3 cells with p53 overexpression treated with ZLN005 compared with PC3 cells overexpressing p53 alone (Fig. 3F). Similarly, the mitochondrial mass of PC3 cells with p53 overexpression treated with ZLN005 was also increased compared to cells with p53 overexpression alone (Fig. 3G). These results indicated that inhibition of PGC-1α may be involved in p53-induced mitochondrial dysfunction.

p53 decreases the expression of genes and proteins associated to mitochondrial biogenesis and fission/fusion by inhibiting PGC-1α. The results of the present study confirmed that activation of PGC-1α by ZLN005, a PGC-1α activator, ameliorated the mitochondrial dysfunction induced by p53. Since PGC-1α regulates mitochondrial biogenesis and fission/fusion, the gene expression levels of NRF1, TFAM, SDHA, MFN1/2 and DRP1 were next examined. The results demonstrated that p53 inhibited the expression of NRF1, TFAM, SDHA, MFN1/2 and DRP1 genes, while ZLN005 partially reversed the inhibitory effect of p53 on these genes (Fig. 4A and B). The expression levels of the corresponding proteins were also examined by western blotting. As revealed in Fig. 4C-F, p53 inhibited the expression of NRF1, TFAM, SDHA, Mfn1/2 and DRP1, while ZLN005 partially reversed the inhibitory effect of p53 on the expression of these proteins. These results indicated that p53 decreased the expression of genes and proteins associated to mitochondrial biogenesis and fission/fusion by inhibiting PGC-1α.

p53/PGC-1α-mediated mitochondrial dysfunction promotes apoptosis of PC3 prostate cancer cells. Peng *et al* (15) revealed that changes in mitochondrial biogenesis and fission/fusion not only affect mitochondrial function but also determine cell survival and death. To investigate the effect of the p53/PGC-1α pathway on PC3 prostate cancer cells, the rate of apoptosis of PC3 prostate cancer cells was examined. Flow cytometric analysis revealed that the rate of apoptosis was decreased in p53-overexpressing cells treated with ZLN005 compared with cells only expressing p53 (Fig. 5A and B). Western blot analysis demonstrated that the expression of cleaved caspase-3 and Bak were slightly decreased in p53-overexpressing cells treated with ZLN005 compared with p53 overexpression alone (Fig. 5C and D). These results indicated that the p53/PGC-1α pathway promoted apoptosis and the apoptosis induced by the p53/PGC-1α pathway may be associated with mitochondrial

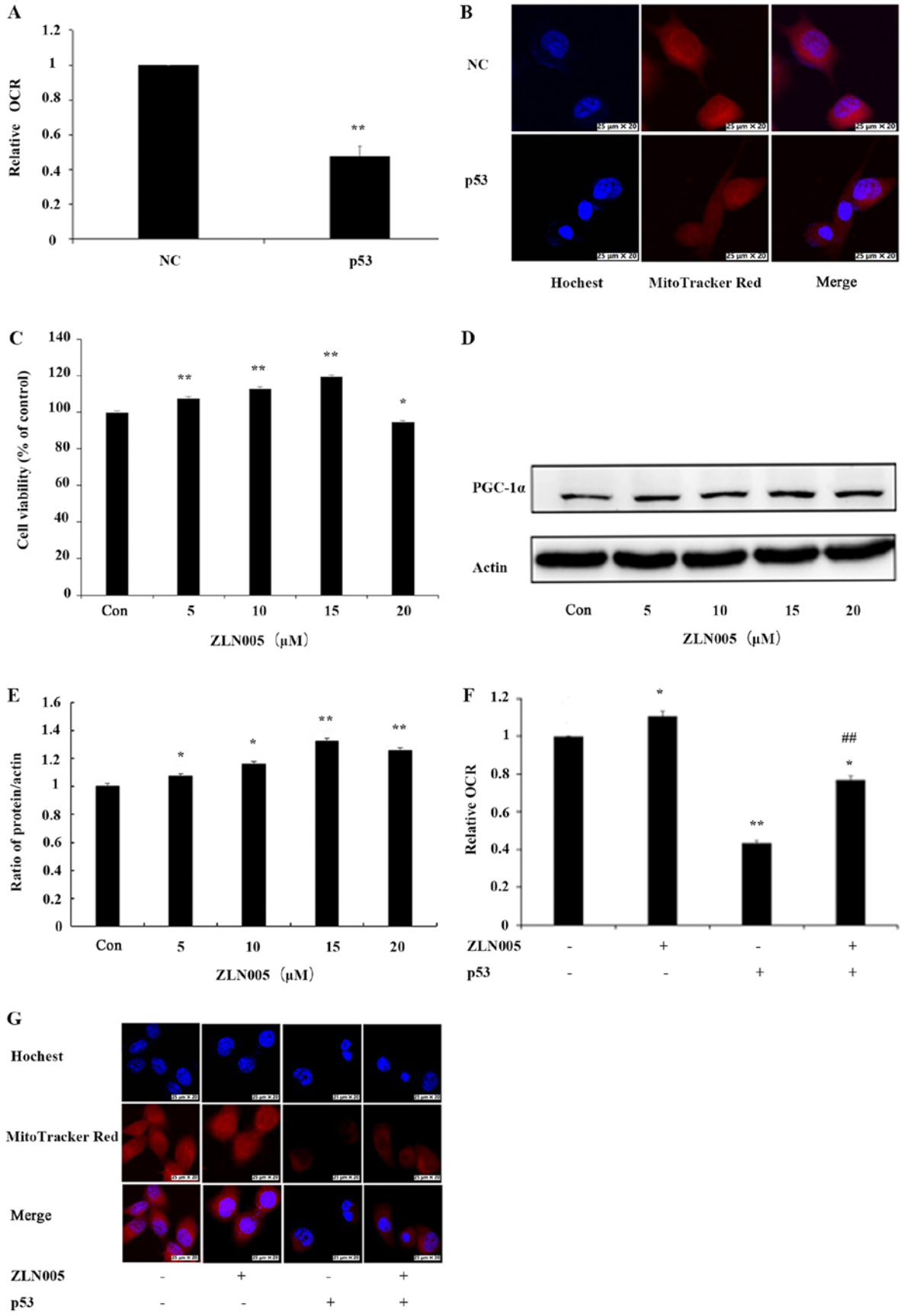

Figure 3. Inhibition of PGC-1α is involved in p53-induced mitochondrial dysfunction. (A) Detection of OCR. Data are presented as the mean ± standard deviation (n=3). **P<0.01 vs. the NC. (B) MitoTracker Red staining for detection of mitochondrial mass. (C) PC3 cells were treated with different concentrations of ZLN005 and cell viability was determined by MTT assay. Data are presented as the mean±standard deviation (n=3). *P<0.05 and **P<0.01 vs. the control. (D and E) PGC-1α expression was analyzed by western blotting in PC3 cells treated with ZLN005 (5, 10, 15, or 20 μM) for 24 h. (F) PC3 cells were transfected with a p53 overexpression construct and treated with/without ZLN005 (15 μM) 4-6 h after transfection. Detection of OCR. Data are presented as the mean ± standard deviation (n=3). *P<0.05 and **P<0.01 vs. the control, $^{\#\#}$P<0.01 vs. p53. (G) MitoTracker Red staining for detection of mitochondrial mass. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; OCR, oxygen consumption rates; NC, negative control; Con, control.

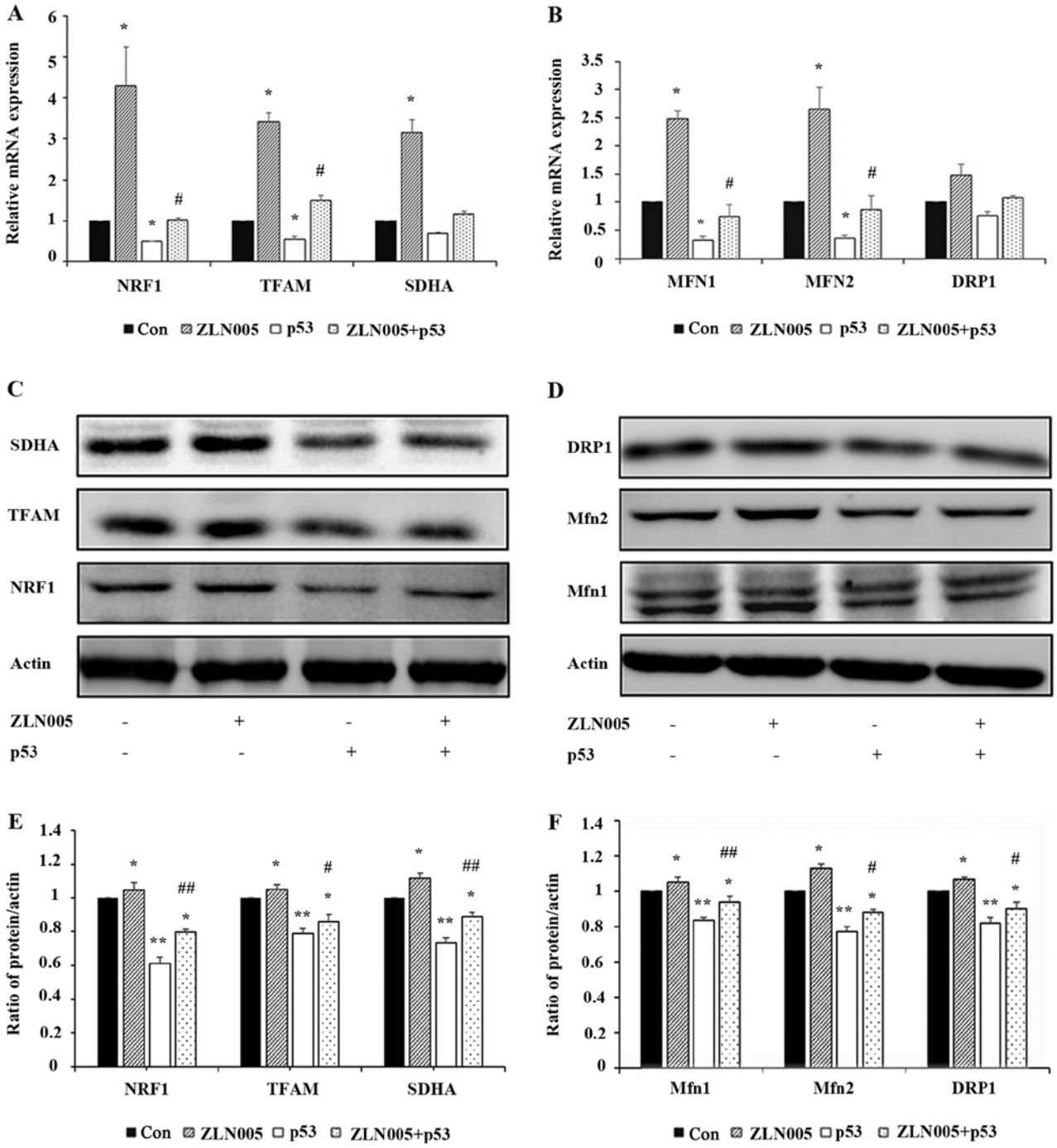

Figure 4. p53 reduces the expression of genes and proteins associated to mitochondrial biogenesis and fission/fusion by inhibiting PGC-1α. The expression levels of (A) mitochondrial biogenesis and (B) fission/fusion genes were detected by RT-qPCR. Data are presented as the mean ± standard deviation (n=3). *P<0.05 vs. the control; $^{\#}$P<0.05 vs. p53. The expression levels of proteins involved in (C) mitochondrial biogenesis and (D) fission/fusion were analyzed by western blotting. Quantitation of (E) NRF1, TFAM and SDHA, and (F) Mfn1, Mfn2 and DRP1 were assessed using ImageJ software. Data are presented as the mean ± standard deviation (n=3). *P<0.05 and **P<0.01 vs. the control; $^{\#}$P<0.05 vs. p53, $^{\#\#}$P<0.01 vs. p53. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; RT-qPCR, reverse transcription-quantitative PCR; Con, control; NRF1, nuclear respiratory factor 1; TFAM, transcription factor A, mitochondrial; SDHA, succinate dehydrogenase complex flavoprotein subunit A; Mfn, mitofusin; DRP1, dynamin-related protein 1.

dysfunction. These findings identified PGC-1α as an essential target of p53-induced apoptosis in prostate cancer cells, indicating that targeting PGC-1α may serve as a new therapeutic strategy for prostate cancer. Consistent with this possibility, western blot analysis demonstrated that knockout of PGC-1α promoted the expression of cleaved caspase-3 in PC3 and DU145 prostate cancer cells and decreased the expression of Bcl-2 (Fig. S1D).

Discussion

The p53 mutation data in prostate cancer from TCGA demonstrated that more than 85% of p53 mutations occur in the p53 DNA binding domain. These mutations not only severely damage the function of the p53 protein, but also reduced the disease-free survival of prostate cancer patients. The present study explored the functional association between p53 and

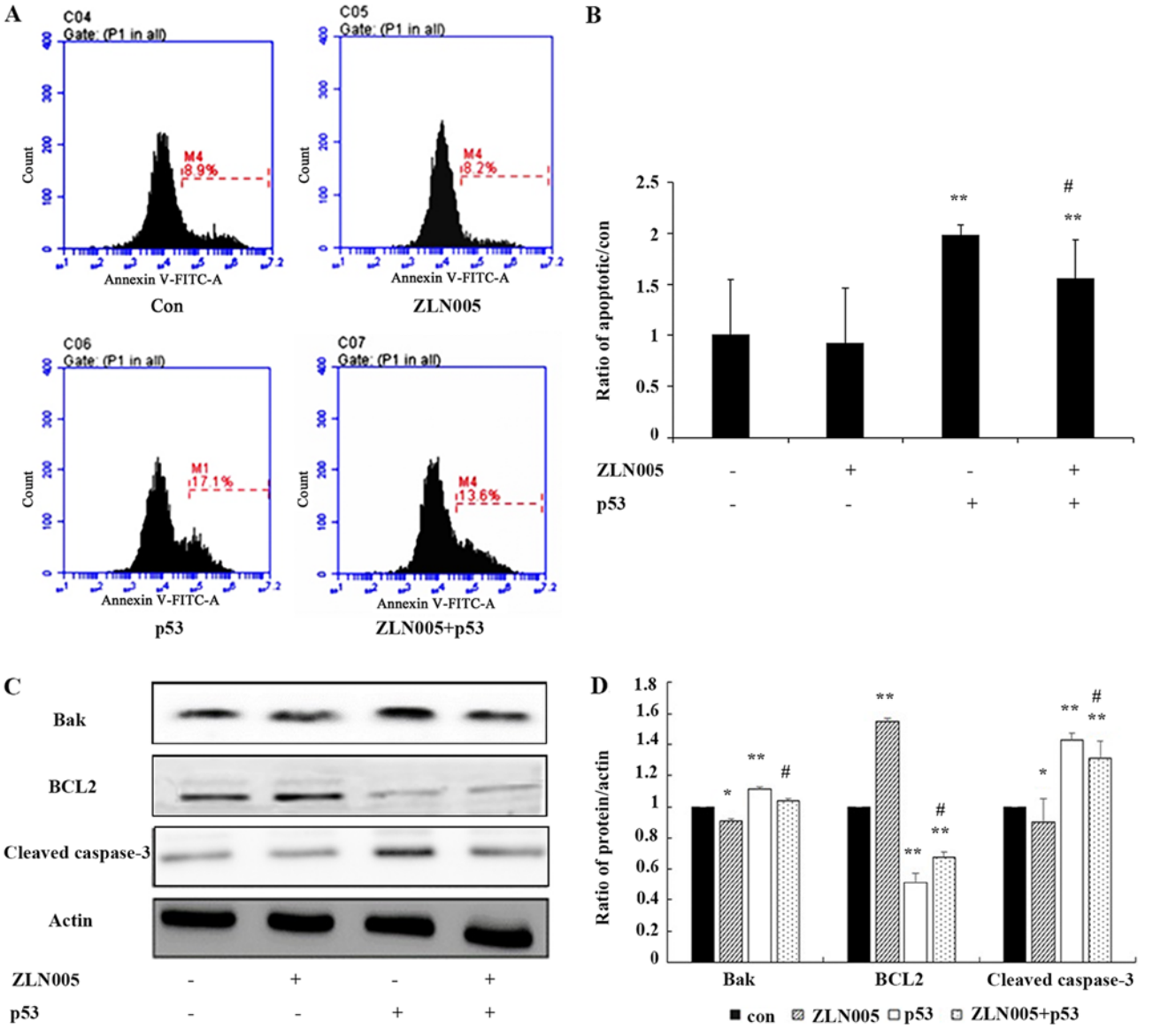

Figure 5. p53/PGC-1α-mediated mitochondrial dysfunction promotes apoptosis of PC3 prostate cancer cells. (A and B) Detection of apoptosis by Annexin V. (C and D) Cleaved caspase-3, Bak and Bcl-2 were analyzed by western blotting. *P<0.05 and **P<0.01 vs. the control; $^{\#}$P<0.05 vs. p53. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; Con, control.

PGC-1α by overexpressing p53 in p53-deficient prostate cancer PC3 cells and revealed that p53 inhibited the protein expression and nuclear localization of PGC-1α. The inhibition of PGC-1α by p53 decreased the expression of genes and proteins associated to mitochondrial biogenesis and fission/fusion and led to mitochondrial dysfunction and apoptosis. These results revealed that PGC-1α was an essential target of p53-induced apoptosis in prostate cancer cells and indicated that targeting PGC-1α may provide a new therapeutic strategy for prostate cancer.

A study reported that overexpression of p53 negatively affects the normal mitochondrial homeostasis in HepG2 cells, but the precise mechanism of mitochondrial dysfunction has not been investigated (21). Another study demonstrated that PGC-1α promotes prostate cancer cell growth by activating the AR. However, whether the survival and death of prostate cancer cells are determined by mitochondrial function regulated by PGC-1α remains unclear (5). The present study demonstrated that inhibition of PGC-1α by p53 induced mitochondrial dysfunction and apoptosis of PC3 cells, indicating an essential involvement of PGC-1α in p53-mediated control of mitochondrial dysfunction and apoptosis. The results not only revealed that inhibition of PGC-1α by p53 is associated with mitochondrial dysfunction but also confirmed the importance of PGC-1α in cell survival.

The present study focused on the regulation of PGC-1α by p53. Previous studies have demonstrated that p53 transcriptionally inhibits and promotes PGC-1α in other non-tumor cells (22,23). For example, p53 binds to the repressive-954 and -564 regions of the mouse PPARGC1A promoter and inhibits the expression of PGC-1α (22,23), However, upon antioxidant glutathione shortage, p53 is released from the two repressive regions and binds to the -2317 region, which is positively associated to increased PGC-1α expression (22). The various activities of p53 on PGC-1α expression may be associated with redox modification of critical p53 amino acids that affect

its DNA binding activity (22). The present study revealed that p53 induced a decrease of PGC-1α protein expression and nuclear localization in PC3 tumor cells. However, Aquilano *et al* (22) demonstrated that p53 binds to the -1237 region in the human PPARGC1A promoter in SH-SY5Y cells to enhance PGC-1α expression. Collectively, these results indicate that p53 exhibits various effects on PGC-1α expression in tumor cells. There are at least two possible reasons for these contradictory activities of p53 on PGC-1α expression in tumor cells. First, post-transcriptional modification of p53 may result in the binding of p53 to different regions of the PGC-1α promoter. It was hypothesized that the p53-binding region in the human PPARGC1A promoter may be the functional homolog of the -954 and -564 regions and not the -1237 region in the mouse PPARGC1A promoter. Second, there may be protein-protein interactions between p53 and PGC-1α. Based on the results of the present study, inhibition of PGC-1α by p53 at the transcriptional level does not fully explain the decreased expression of PGC-1α in the nucleus. Protein-protein interactions between p53 and PGC-1α may explain PGC-1α reduction in the nucleus and this possibility will be the focus of a future study.

The present study identified that activation of PGC-1α by ZLN005 resulted in amelioration of the mitochondrial dysfunction and apoptosis induced by p53. Activation of PGC-1α by ZLN005 regulated mitochondrial biogenesis and fission/fusion, which is probably involved in the maintenance of mitochondrial function and promotion of cell growth and survival. Although previous studies have demonstrated the effects of ZLN005 in increasing the expression of PGC-1α, further experiments to evaluate the effect of PGC-1α overexpression are required, because ZLN005 also transcriptionally promotes genes encoding the deacetylase SIRT1 and antioxidant enzymes SOD1 and HO-1 (24-26). Whether activation of these genes is involved in improving mitochondrial function and apoptosis is unclear.

In conclusion, the findings of the present study demonstrated that p53 decreased the expression of mitochondrial biogenesis and fission-/fusion-associated genes by inhibiting PGC-1α, leading to mitochondrial dysfunction and ultimately apoptosis. The results revealed a pro-cancer effect from PGC-1α and indicated that PGC-1α may be a new therapeutic target for PC3 prostate cancer cells. However, the precise regulatory mechanism linking p53 and PGC-1α remains to be elucidated and requires further investigation.

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Availability of data and materials

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

Authors' contributions

LS, YL, JS, JL and YNL designed the study. JL and LC collected and analyzed the data. JL, YX, RG and BY performed the experiments. JL drafted the manuscript. YNL, JS and YL reviewed the manuscript. All authors reviewed the manuscript and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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